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FT food technology

OCTOBER, 1960
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CALL FOR NOMINATIONS

The Babcock-Hart Award

Any member of the Institute of Food Technologists may submit a nomination for the 1961 Babcock-Hart Award between now and the January 1, 1961 deadline.

Nominations must be made on the prescribed nomination form which may be obtained from the Executive Secretary, in order to be considered.

Donor of the $1,000.00 and engrossed plaque accompanying the Award is the Nutrition Foundation. Purpose of the Award is: "To honor a person who has distinguished himself or herself by contributions to Food Technology which have resulted in improved public health through some aspect of nutrition or more nutritious food."

A nominee, to be eligible, must have made a technological development, or a scientific contribution leading directly to a technological development, which favorably affects the nutritional well-being of the public. **Contributions in clinical nutrition and fundamental scientific studies in nutrition cannot be considered as qualifying according to conditions established by the Donor.**

The Nicholas Appert Award

Any member of the Institute of Food Technologists may submit a nomination for the 1961 Nicholas Appert Award between now and the January 1, 1961 deadline.

Nominations must be made on the prescribed nomination form which may be obtained from the Executive Secretary, in order to be considered.

Donor of the $1,000.00 honorarium accompanying the Award is the Institute of Food Technologists. The Award, originated by the Chicago Section, also includes a Bronze Medal which is donated by that Section. Purpose of the Award is: "To honor a person for pre-eminence in and contributions to the field of Food Technology."

Qualifying criterion for a nominee includes merely the fact that the nominator consider the individual so pre-eminent in the field of Food Technology that he or she should be acclaimed for contributions thereto. This precludes consideration of personality or other characteristics outside the field of contributions to Food Technology, as these factors may not be considered by the Awards Jury in making the selection.

Call for Nominations—Food Technology Achievement Award

The IFT Food Technology Achievement Award is given annually to recognize and honor an outstanding food process and/or product, which represents a significant advance in the application of Food Technology to food production, and which has been successfully applied in actual commercial operation for at least six months but not over four (4) years prior to the date of calling for nominations. Insofar as possible, this Award shall be personalized by public recognition of the individuals(s) responsible for the achievement.

The Award will include the following: to the Company or Institution, a bronze Plaque. To the individual(s) judged to have made major contributions to the achievement, either through basic research or development, public recognition at the time of the presentation of the Award, and an engrossed Plaque.

Nominations for this Award may be made by any member of the Institute of Food Technologists. Each member of the Institute is urged to give serious consideration to the nomination of a product or process, not necessarily one in which your company is concerned. Each nomination must be in letter form and include the following:

A. Name of Company or Institution.
B. Name of Product and/or Process.
C. Description of Product or Process.
D. Statement of reason for considering this a meritorious achievement.
E. Statement listing individuals chiefly responsible for the achievement and their individual contributions.
F. Statement indicating the time and extent of commercial utilization.

NOMINATIONS MUST NOT EXCEED THREE, SINGLE-SPACED, TYPEWRITTEN PAGES.
Nominations should be sent, **postmarked no later than December 1st, to:**

INSTITUTE OF FOOD TECHNOLOGISTS
176 W. Adams Street
Chicago 3, Illinois

October Cover. Processing the Japanese mandarin orange. Typical worker (upper left) picking "Mikan" in a Japanese orchard. The fruit, after arrival at the processing plant, undergoes size-grading and blanching in hot water, after which outer peels are removed by hand, and segments separated, also manually. Outer skins of the segments are then dissolved in acid and alkali. The outlet side (upper right) of the alkali treatment drums is depicted, as well as the soaking in cold running water and draining of the peeled segments. The size-grading method (lower left) used is the next step shown, and this is followed by (lower middle) filling, syruping, sealing, cooking, cooling, and labelling. Information and photographs for this salute to the Japan Section were supplied by Dr. Masao Fujimaki, University of Tokyo.

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(2) A food container component
(3) An equipment lubricant
(4) An emulsifier in food."

We have prepared Bulletin A-1 to give general information on this subject; included are a list of 69 pertinent scientific papers and patents and a compilation of many suggested uses. These range from slab oils to shortenings, and include coatings for foods as well as plasticizers for other coating materials.

Other bulletins are now available which give information on specific applications:

A-2. Poultry
A-3. Frozen and Processed Meats
A-4. Frozen Fish
A-5. Dried Fruits
A-6. Nuts and Candies
A-7. Storage Eggs

Food processors are welcome to copies of these bulletins, as well as samples of different types of *MYVACET Distilled Acetylated Monoglycerides.*

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Committees of the Institute of Food Technologists for the 1960-61 Year

Committee on Awards

This committee is responsible for the administration of the Awards program of the Institute in accordance with the policies, rules and regulations governing awards as amended to May, 1960.

Dr. Horace Sipple, Chairman, The Nutrition Foundation, 99 Park Avenue, New York 16, New York

Dr. K. G. Weckel, Vice Chairman, Babcock Hall, University of Wisconsin, Madison, Wisconsin

Nicholas-Appert Award Jury: Dr. H. E. Robinson, Chairman, Swift and Company, Union Stock Yards, Chicago, Illinois

Babcock-Hart Award Jury: R. B. Wakefield, Chairman, Gerber Products Company, Fremont, Michigan

Food Technology Achievement Award Jury: Dr. P. K. Bates, Chairman, Carnation Company, 8015 Van Nuys Blvd., Van Nuys, California

International Award Jury: Dr. E. M. Mrak, Chairman, University of California, Davis, California

Graduate Awards Jury: Dr. R. E. Buck, Chairman, Head, Food Tech. Res. Lab., H. J. Heinz Company, Pittsburgh 30, Pennsylvania

Undergraduate Awards Jury: Dr. John M. Jackson, Chairman, American Can Company, R & D Center, Barrington, Illinois

Committee on Citrus Products

The objectives of this committee are as follows:

1. This committee in conjunction with the Annual Meeting Program Committee will establish a pattern for the presentation of papers on citrus research and technology at the annual meeting of the Institute.
2. Continue sub-committee work on methodology aiming toward standard procedures for use by state and federal agencies.

R. E. Cox, Chairman, 404 E. Genesee Avenue, Los Angeles 36, California

Robert W. Kilburn	R. E. Cox
W. R. Channell	Dr. W. R. Roy
Homer W. Holton	Dr. Matthew K. Valdhuis
Ellis M. Byer	Dr. Fred Wenzel
Omer R. McDuff	Williard E. Baier
Howard Trumm	Dr. Francis Griffiths
Dr. E. A. Beavens	Dr. L. G. MacDowell
C. G. Beisel	Virgil S. Troy
Charles Brokaw	Dr. Reese Vaughn

Committee on Civil Defense

The primary objective of this commitee is to create an awareness in the members of the Institute of Food Technologists of the problems that could be faced by the food industry in case of a national emergency.

Dr. Richard H. Forsythe, Chairman, Department of Poultry Husbandry, Iowa State University, Ames, Iowa

Dr. Robert Baldwin	Dr. Frank L. Gunderson
Dr. Franklin Barber	John H. Kilbuck
Dr. Howard Bauman	Dr. E. M. Mrak
Dr. Rudy H. Ellinger	Dr. Roy C. Newton
William Ferracone	George W. Putnam

Dr. Keith Lewis

Committee on Constitution and By-Laws

The committee shall receive proposals for amendments to the Constitution. These it shall study and make recommendations thereon for submission to the Executive Committee of the Council.

Dr. Byron Shinn, Chairman, Armour and Company, Union Stock Yards, Chicago, Illinois

Dr. R. P. Joslin	R. F. Light
Radcliffe F. Robinson	Hugh Griswold

C. D. Pratt

Committee on Education

Objectives:

Continue work with schools in curriculum planning to bring all curricula closer to ideal objective of the Institute.

Recommend to the Institute actions which should be taken to further interest students in the field of Food Science, Technology and Engineering.

Dr. Robert M. Schaffner, Chairman, Libby, McNeill & Libby, Chicago 4, Illinois

Dr. Gale Ammerman	Dr. R. T. Milner
G. E. Brissey	Dr. E. B. Oberg
Dr. Irving S. Fagerson	D. L. Schott
J. M. Heinen, Jr.	Dr. H. W. Schultz
Dr. Melvin Johnston	Dr. B. S. Schweigert
Varnum T. Ludington	Dr. K. G. Weckel

Dr. Virgil T. Wodicka

Committee on Finance

This committee has the general responsibility of initiating policy in respect to the handling of Institute finances, of approving the annual budget, and of discharging such other duties in connection with the business affairs of the Institute as may be assigned to it by the Treasurer.

Dr. A. N. Prater, Chairman, Gentry, Inc., 1111 South Central Avenue, Glendale 4, California

W. Ray Junk	Dr. James W. Evans
Dr. John M. Jackson	Stephen L. Galvin

Committee on Food Additives and Components

General Objective:

To collect and interpret information of both a regulatory and scientific nature on new developments and problems in the field of food additives and standards of identity as may be decided by the committee to be needed and helpful to the Institute of Food Technologists. Such material will be ordinarily disseminated through publication in FOOD TECHNOLOGY.

Dr. John F. Mahoney, Chairman, Merck & Company, Rahway, New Jersey

Dr. H. W. Schultz	C. D. Pratt
R. Thiessen	Dr. Margaret Ives
Dr. R. L. Hall	Dr. R. C. Sherwood
Dr. D. B. Hand	Walter H. Williams

Committee on History

This committee's objective for this year is:

Continue work toward completion of history on—

a. IFT Awards
b. History of Regional Sections
c. History of IFT publications

Dr. E. A. Beavens, Chairman, U.S.D.A., 263 Chester Avenue, Pasadena 5, California

Dr. Samuel C. Prescott	Miss D. A. Huber
Dr. W. V. Cruess	Dr. J. H. Nair

Dr. J. G. Woodroof

Committee on Membership

This Committee is to work with the Executive Office in initiating ways and means to increase Institute membership throughout the world.

Warren R. Schack, Chairman, Canned Meats Research Division, Swift & Company, Union Stock Yards, Chicago 9, Illinois

Rees B. Davis	Alfred P. Rumminger
Dr. D. C. Kuesel	Elverton B. Stark

Committee on Nominations

The nominees for officers and councilors-at-large shall be selected by the Committee on Nominations as prescribed in Article VIII, Section 1, 2 and 3 of the Constitution and Article IX, Section 7 of the By-Laws.

Dr. Gale Ammerman, Chairman, Libby, McNeill & Libby, Central Food Research Laboratory, Blue Island, Illinois

Dr. R. E. Hein	H. B. Rogers
H. T. Griswold	Clarence W. Dubois

The Chairman of each regional section is a member of this Committee in addition to the above.

Program Committee

This permanent committee with rotating membership is responsible for programming for annual meetings of the Institute. Its objective shall be continued improvement of programs to attract the presentation of the finest research and advanced techniques in Food Science, Technology and Engineering.

Fred C. Baselt, Chairman, American Can Company, 100 Park Avenue, New York 17, New York

Dr. P. A. Buck	Mrs. Lois A. Sather
Dr. Betty Watts	Dr. Paul E. Ramstad
Dr. H. E. Goresline	M. J. Copley
Dr. J. J. Albrecht	Dr. R. C. Sherwood
J. B. Anderson	Dr. Hans Lineweaver
Dr. Edward A. Nebesky	Dr. Amihud Kramer
R. Thiessen, Jr.	Dr. John F. Murphy
Dr. B. F. Daubert	Dr. John Jackson

Dr. J. F. Mahoney

Committee on Publications

"This Committee has the responsibility and authority to investigate, review, study and recommend on any matter concerning publication policy to the Executive Committee of the Council," Article IX, Section 6—By-Laws.

Dr. Howard Spencer, Chairman, Dow Chemical Company, Midland, Michigan

Dr. George Beach	Dr. Reid Milner
Dr. S. A. Goldblith	Dr. Georg A. Borgstrom

Dr. B. F. Daubert

Committee on Qualifications

This committee shall operate in accordance with Article IX, Section 4 of the By-Laws of the Institute.

George E. Brissey, Chairman, Swift & Company, Union Stock Yards, Chicago 9, Illinois

Henry P. Furgal	Dan J. Ridenour
Dr. C. S. Miner	John M. Heinan, Jr.

F. Warren Tauber

Foreign Aids:

Walter McAllister	Dr. Victor M. Lewis

Lincoln J. Frost

Committee on Quality Control of Food Products

Objective:

Concentrate on forming a strong Quality Control program for the 1961 annual meeting to be held in New York, May 7–11.

Bruno Filice, Chairman, Filice and Perrelli Company, 1200 South 10th Street, Richmond, California

Gerald Desmond	Dr. Amihud Kramer
Leonard Gieseker	E. Norman Liles
Dr. Richard Guyer	Rolie O'Neal
William J. Hoover	F. Miles Sawyer
Stanley Hotchner	Dr. Howard Stier
Harry Korab	Hugo Wistreich

Dr. Oliver J. Worthington

Committee on Resolutions

The responsibility of this committee is to prepare appropriate resolutions and present them at the Annual Meeting as authorized by the Executive Committee and the Council.

A. J. Ganz, Chairman, 604 West 11th Street, Wilmington, Delaware

H. A. Barnby	Norman H. Ishler

R. H. Johnson

Committee on Taste Testing

General Objectives:

To list and classify procedures and methods used in taste testing.

To evaluate procedures and methods used in taste testing and recommend those that should be adopted for use.

To encourage research effort in those areas lacking definitive information.

Dr. H. L. Stier, Chairman, Division of Statistics, National Canners Assoc., 1133 20th Street, N. W., Washington 6, D. C.

Dr. Dean Foster	Dr. Elizabeth Murphy
Dr. M. A. Amerine	Dr. D. R. Peryam
Dr. R. J. Carbonell	Prof. Lois A. Sather
John Coleman	Dr. Miles Sawyer
Miss Elsie Dawson	Miss Marion Simone
Dr. Amihud Kramer	Dr. K. G. Weckel

Ad Hoc Committee to Study Ways and Means of Advancing the Science of Food Technology

This committee is to study areas and ways in which Industry can best serve to advance the Science of Food Technology. Report to the Executive Committee at its mid-year meeting.

Dr. H. W. Schultz, Chairman, Oregon State College, Corvallis, Oregon

H. T. Griswold	Dr. Carl H. Krieger
Howard P. Milleville	R. B. Wakefield

Ad Hoc Committee to Study Employment System and Procedures

Objectives:

1. To study the present activities of the Institute in the area of employment and recommend an improved service, if such is possible.
2. Evaluate the employment activities at annual meetings and draw up a procedure to be followed to insure more effective service to members of the Institute. This procedure to be adopted for 1961 annual meeting.

Charles W. Kaufman, Chairman, National Dairy Products, Research & Development Division, Glenview, Illinois

William X. Clark	Clifford F. Evers
Dave Eolkin	Richard Van Fleet

C. K. Wiesman

Ad Hoc Committee to Study the Advisability of Establishing a Code of Ethics for Institute Members

The objectives of this Committee is to investigate, study, and recommend to the Executive Committee whether or not a Code of Ethics should be adopted covering members of the IFT.

Dr. H. W. Schultz, Chairman, Oregon State College, Corvallis, Oregon

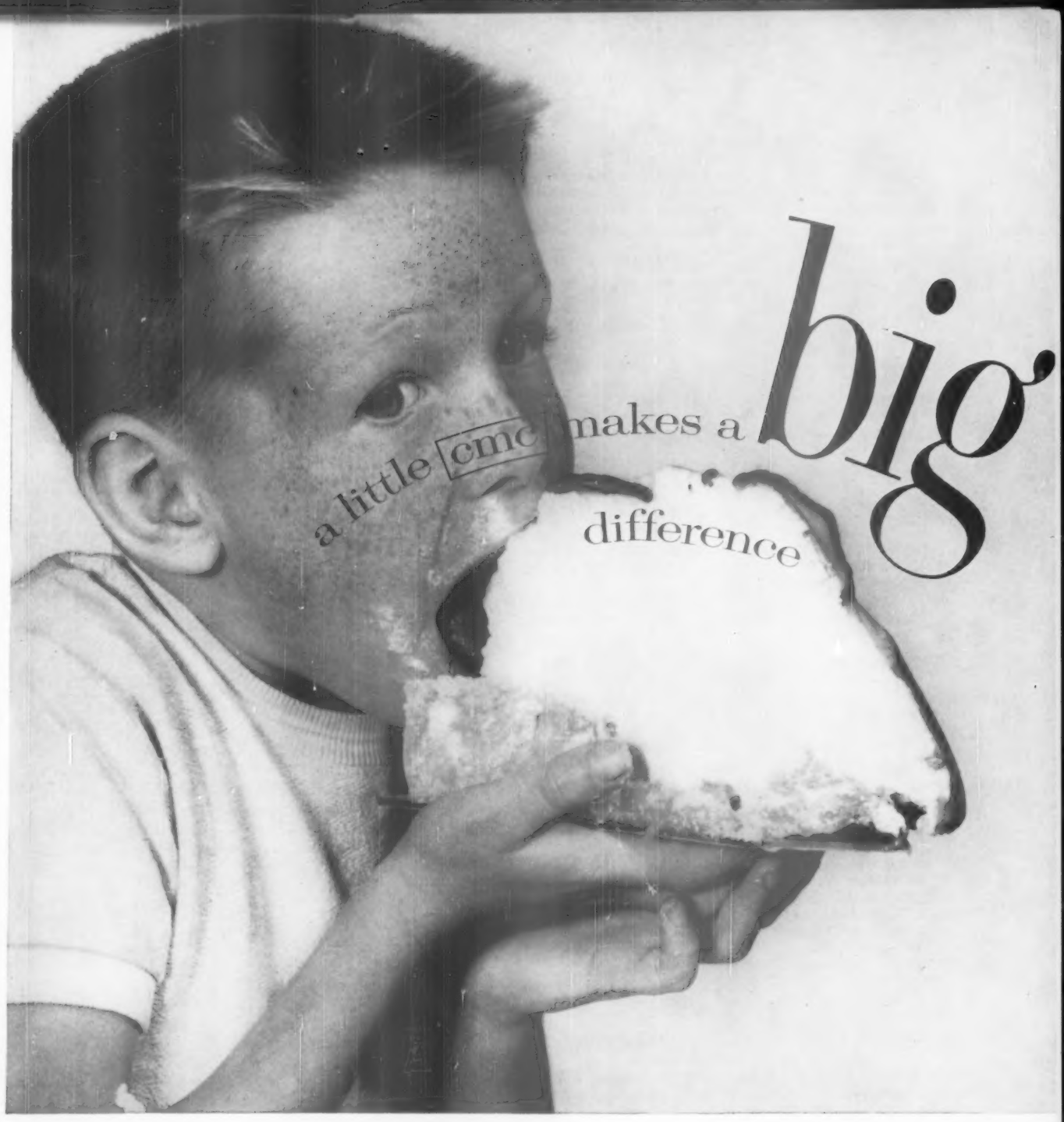
A. J. Ganz	Dr. F. N. Peters
R. B. Marshall	Dr. P. K. Bates
Hamilton W. Putnam	Dr. R. H. Vaughn

Ad Hoc Committee on International Congress on Food Technology

This committee is to work with the Society of Chemical Industry in formulating a program for the 1962 International Congress on Food Technology.

Dr. E. M. Mrak, Chairman, University of California, Davis, California

Dr. George Stewart



If the proof is in the testing, here's a young fellow who's obviously had a point proven to him. Chances are that Hercules® CMC—Cellulose Gum—has been used in the formulation because Cellulose Gum is becoming increasingly popular as a stabilizer for both pie fillings and meringues.

In lemon pie fillings, such as the delectable one in the picture, Cellulose Gum is providing greater clarity, elimination of cracking and syneresis, and a more pleasing, less short texture.

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Virginia Cellulose Department

HERCULES POWDER COMPANY

Hercules Tower, 910 Market Street, Wilmington 99, Delaware

VC60-2

Ad Hoc Committee to Develop A Manual for Section Officers

Objective:

To develop a manual which will assist all section officers by giving them the duties and responsibilities of the various offices along with recommended committees and their duties.

Dr. H. A. Campbell, Chairman, General Foods Corporation, 555 South Broadway, Tarrytown, New York

F. J. Erickson — Dr. J. H. Nair
Dr. Frank A. Lee — Hamilton W. Putnam
Radcliffe F. Robinson

Ad Hoc Committee on Public Relations

Objectives:

1. Investigate the possibility of securing a Public Relations firm to outline a program of public relations for the Institute.
2. To determine the cost of the above outlined program.

Dr. Loren B. Sjostrom, Chairman, A. D. Little Company, Cambridge, Massachusetts

Dennis H. Murphy — Norbert J. Leinen
Robert D. McMillen

Liaison Representative to the Agricultural Research Institute of the National Research Council

Appointed by the President to keep the Institute informed of the activities of this organization. An annual report to the Council should be made.

Dr. Ira Somers, Director, Research Laboratories, National Canners Association, 1133 20th Street, N. W., Washington 6, D. C.

Liaison Representative to the Council of American Association for the Advancement of Science

These men serve as representatives of the Institute of Food Technologists on the coordinating committee of the American Association for the Advancement of Science and report to the Council transactions of interest to the Institute.

Dr. Charles N. Frey, 45 Cambridge Road, Searsdale, New York

Dr. M. A. Joslyn, Department of Food Technology, University of California, Berkeley 4, California

Liaison Representative to the Food and Nutrition Board of the National Research Council

All appointments to the Food and Nutrition Board are made by the Chairman of the National Research Council. At the invitation of the National Research Council, the President of the IFT nominates a member for consideration and appointment as Liaison Representative. This appointment is for an indefinite period, but like all committee appointments is subject to annual reaffirmation by the President of the Institute. An annual report is made to the Council.

Dr. L. J. Teply, Chief, Expanded Aid to Nutrition UNICEF, United Nations Building, New York, New York

Liaison Representative to the Food Protection Committee of the National Research Council

Appointed by the President to keep the Institute informed of the activities of this organization. An annual report will be made to the Council.

Dr. John F. Mahoney, Merck & Company, Inc., Rahway, New Jersey

Liaison Representative to the U. S. National Committee for the International Institute of Refrigeration

This appointment to the Committee for the International Institute of Refrigeration is made by the National Research Council. At the invitation of the National Research Council, the President of the IFT nominates a member for consideration and appointment is for a three-year term starting July 1, 1959. An annual report is made to the Council.

Dr. H. C. Diehl, Managing Director, Trans-American Refrigerated Services, Ltd., P. O. Box 1275, Colorado Springs, Colorado

N. CALIFORNIA

The high point of our August 18th meeting, held at the Far East Cafe in San Francisco's famed Chinatown, was the most interesting "Travel Excerpts about Places and Foods," presented informally to this attentive audience by Mr. Milton P. Duffy, Chief of the Bureau of Food and Drug Inspection, State of California. Mr. Duffy had just returned in May from an extensive world tour.

The Port of Spain, Trinidad, was the first stop of their luxury liner. They were greeted by Calypso music. This island has sugar, cocoa, nutmeg and spices; the latter are protected by shade trees from the hot sun.

Mr. Duffy spoke of the odor of coffee as you land in Rio de Janiero; of the sun-proofed Standard Oil building; of the bank that had no front door. In the "outer" part of Rio are to be found small factories as well as leopards, boars, and monkeys. This city is noted for its aquamarines and other semi-precious stones that are a tourist attraction.

At Bombay, India, a high point was a visit to the Taj Mahal. In Bombay every drug store has a doctor; here can also be seen the turkey fish that really looks like a turkey! Tapioca is found growing like marijuana. It is made into flour and paste and is used in making candy.

On the Cape of Good Hope there are thousands of baboons as well as zebras that run for miles. Several ports were visited while the liner plied the Indian Ocean. Among these was Durban, one of several cities where chutneys could be found. At Durban there is a race track that goes over the highway.

At Zanzibar there is the strong odor of cloves—they are protected here by the shade of the palms. Here, too, cardamon can be found growing like peanuts; various other spices are found here as well as carrots, mangoes, greens and rugs. Modern well-equipped dairies get their milk from the water buffalo as the cow is considered sacred. Vegetables grown and marketed here are larger than those in the states; much poverty, too.

Ceylon has spices and tea, the latter of the camellia family. There are rubber plantations here, and the markets have only native foods.

From Ceylon Mr. Duffy's trip took him through the China Sea to Singapore (Chance Alley where one can buy everything cheap), Malay, through the Gulf of Siam to Bangkok (with marble and jade palaces as well as solid gold Buddhas and silks), Bataan, Manila, Kobe, Tokyo, Honolulu, then to Long Beach and back through the Panama Canal to New York.

The evening's business also included a discussion of the Cruess Award and changes that are to take place in its size and scope.

The traveling gold golf trophy was presented to Mr. Dave Eolkin of Gerber's. Recipients in tournaments past included both Mr. Lloyd Van Auken, also of Gerber, and Mr. John Kilbuck, who is with Stange. Our next regular meeting will be October 20th.

—Shirley V. Noland

SPICE
IS
SPICE
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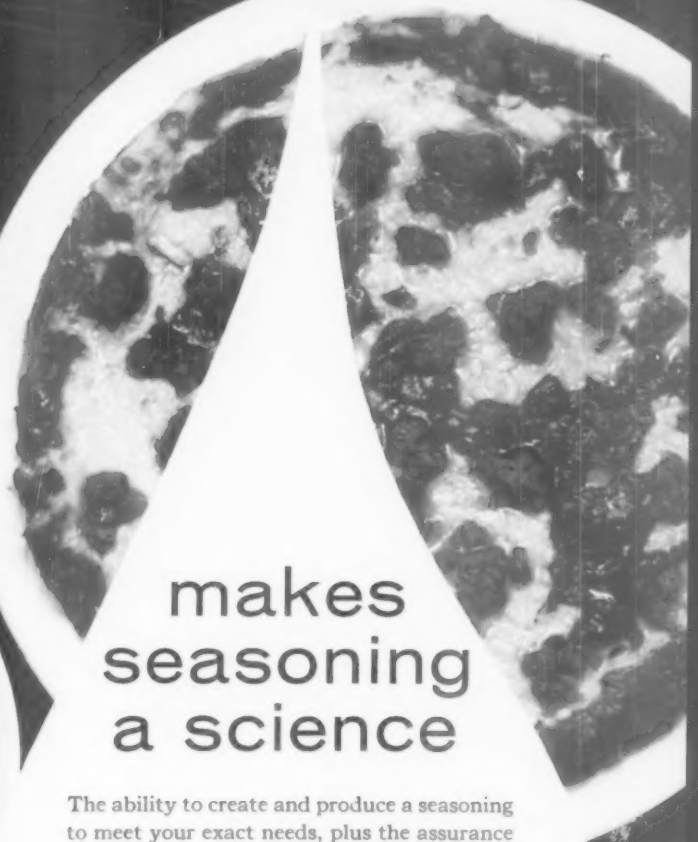

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IN THIS ISSUE–

TECHNICAL ARTICLES IN BRIEF

Food technologist training

The food technologist—his training. Ernest H. Wiegand.

The food technologist is destined to assume an increasing role in our ever growing food industry. With the rapidly changing pattern of this industry trained graduates from our universities will be in great demand. However, it behooves us to see that our graduates are properly trained in the fundamental sciences and engineering that underlie food technology. We must recognize and accept the fact that industry will be critical and will demand that the men they employ are able to get along with their fellow man, assume their obligations as citizens, express their conclusions and convictions clearly, and have a faculty for diplomacy, tolerance and consideration. Technical training is not alone sufficient for the business or professional man. The training of the food technologist will eventually culminate in a five year course. However, the present four year course, if properly developed for the student who does not wish to become a specialist, is adequate to meet his needs, so that he may be able to assume a position in one of several phases of the food manufacturing industry. The number of men able to undertake specialized training will be limited, but it is the privilege and responsibility of the university or college to see that those who can contrbute most are properly prepared. (See page 473).

Core sampling methods *Tuna*

Chemical composition of raw, precooked, and canned tuna. I. Core sampling methods. Clarence J. Carlson, Claude E. Thurston, and Maurice E. Stansby.

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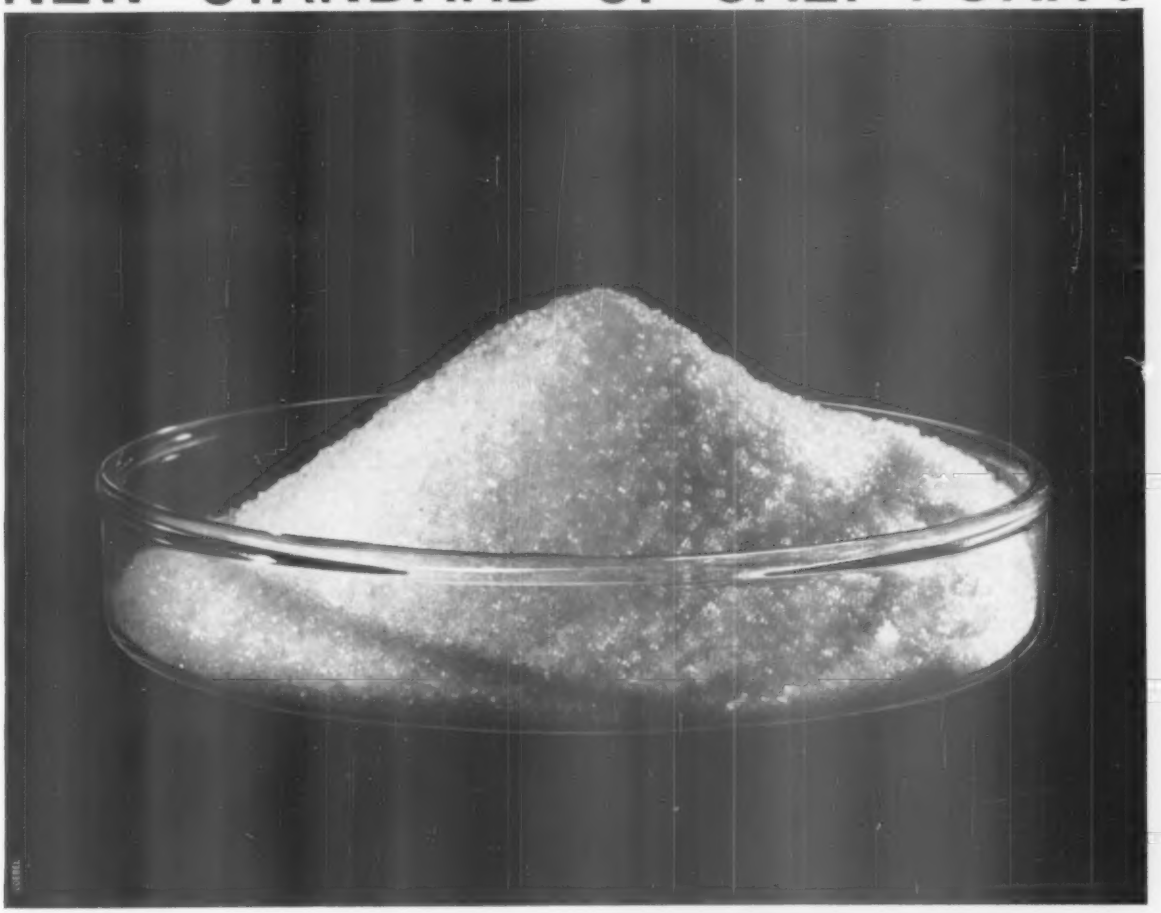

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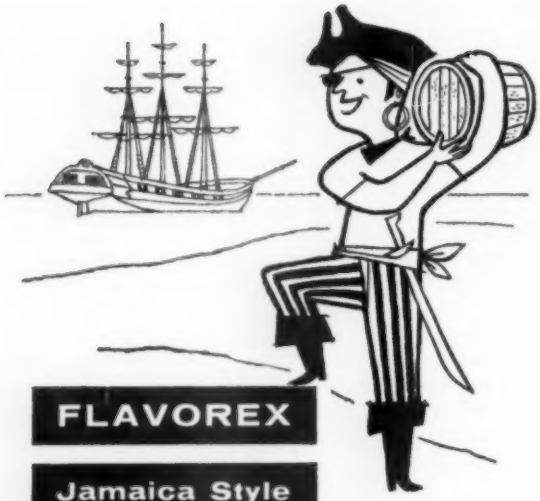

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Enzymatic browning *Effect of inhibitors on apple tissue*

Effect of borates and other inhibitors on enzymatic browning in apple tissue. II. Mechanism. Karakian Bedrosian, M. P. Steinberg, and A. I. Nelson.

The mechanism by which borates inhibit enzymatic browning of apple slices was investigated. Both catechol and a purified polyphenol extract of apple were used as substrate and a commercial phenol oxidase enzyme preparation was used as the catalyst. The reaction was allowed to proceed for 30 minutes at 25° C and the optical density measured at 430 mme. The point of maximum enzyme activity was at pH 7. Borate inhibition was increased by increasing pH of the system and by increasing borate concentration. Sodium tetraborate and boric acid gave closely similar results as inhibitors as did catechol and polyphenol apple extract as substrate. Increasing amounts of catechol added to a boric acid solution caused successive reductions in pH. Borate inhibited oxidation of catechol in absence of enzyme. Addition of borate caused a decrease in the absorption by catecheol of ultraviolet light. Based on this evidence it was concluded that borate inhibits the browning reaction by forming a complex with the substrate and thus preventing oxidation. (See page 480).

Apparatus to study dry-heat resistance of microorganisms

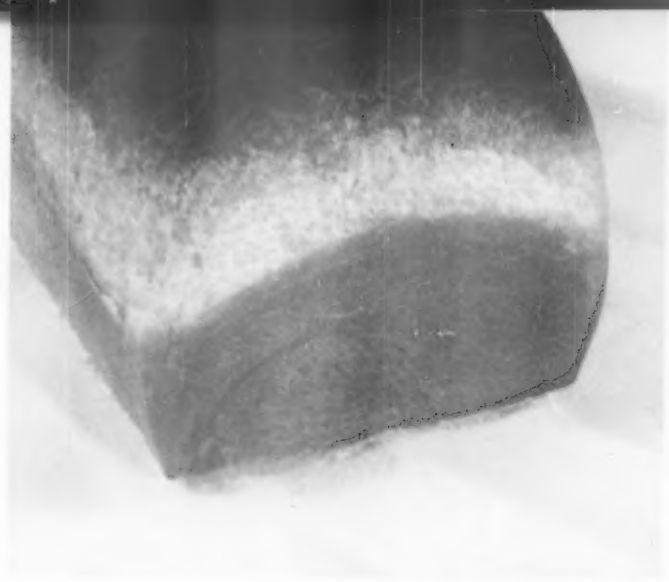
Thermal resistance of microorganisms to dry heat: design of apparatus, operational problems and preliminary results. I. J. Pflug.

An apparatus is described that makes possible the study of the dry-heat resistance of microorganisms under controlled conditions of time and temperature in the range 300 to 380° F (149 to 193° C). The accuracy of the temperature control system is ± 1.0° F and time ± 0.1 minute. The lag correction factor of the sample cups has been evaluated as a function of gas flow for both air and superheated steam. The dry-heat resistance of spores of organism 5230 (similar to *B. subtilis*) to superheated steam has been determined and it was found to have an $LD50_{2.0}$ of 2.4 minutes and a z of 42° F. (See page 483).

Food additives *Flavoring ingredients*

Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment. Richard L. Hall.

The action taken by the Committee of the Flavor Extract Manufacturers' Association on approximately 1000 flavor ingredients is reported and an alphabetized list showing the specific action is provided. (See page 488).



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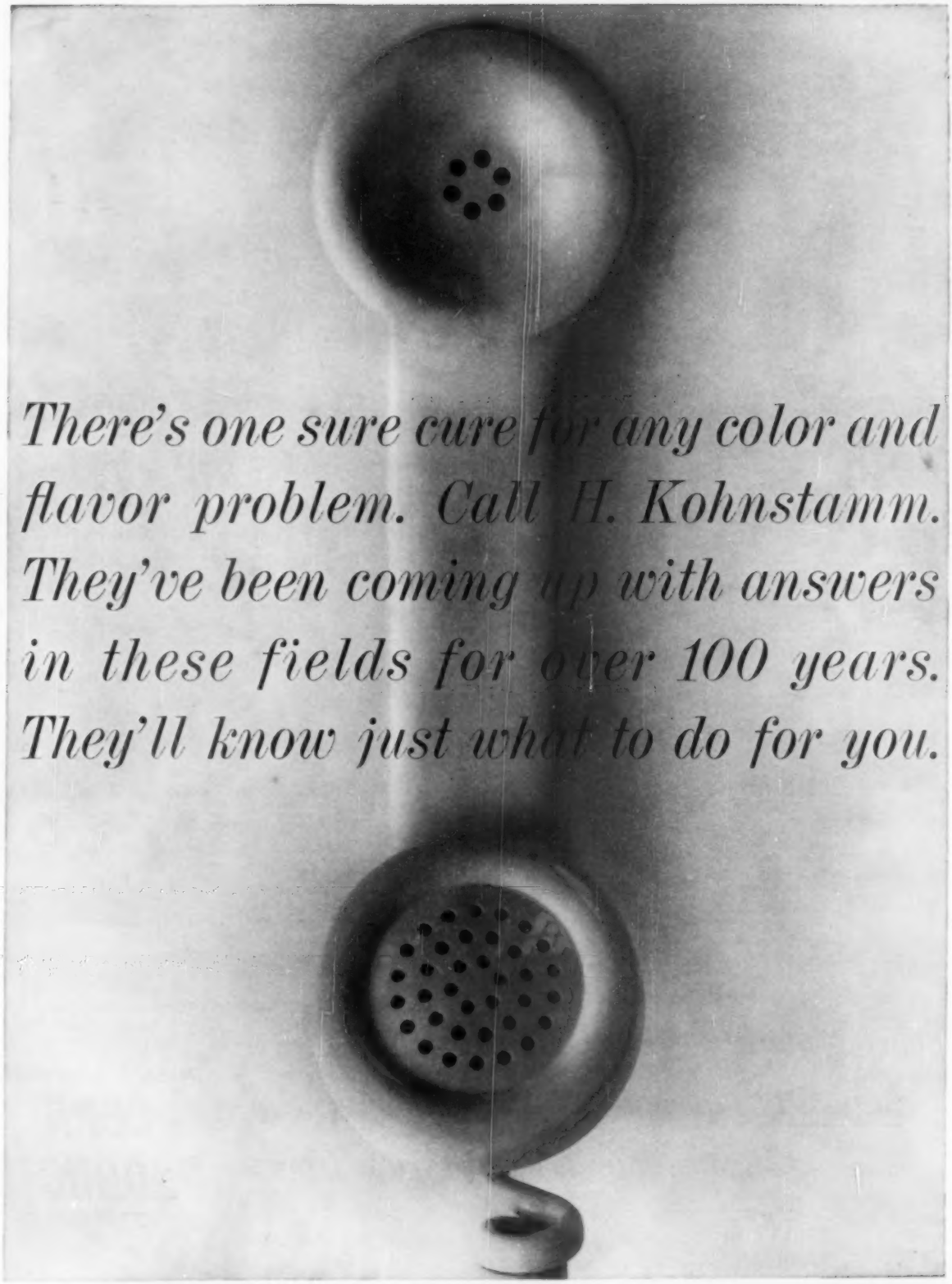
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The Food Technologist—His Training*

Ernest H. Wiegand
Appert Medalist, 1960

FOR THE HONOR bestowed on me as this year's Nicholas Appert Medalist, I wish again to thank the members of the Institute.

It was suggested that an address was in order, on some subject of interest to this group, and on one with which I have been closely associated. In the present ever growing and changing pattern of the food industry, training the food technologist in the science, technology, and engineering involved in the newer developments is becoming more and more important. I have, therefore, chosen the subject, "The Food Technologist and His Training," because of its great interest today and because of my long and close association with this educational field.

During the years that your speaker has been associated with food technology, it has been undergoing rapid changes. This has been especially true in the last 30 to 40 years, and it is mainly due to a more enlightened industry, to changing laws, and to the body of knowledge made possible by a more extensive background of research.

Ernest H. Wiegand

Fifty years ago, food preservation was still carried on in a very crude manner. Much of the work of preparing products in the canning plants was done by hand and was slow and often quite crude. Fortunately, labor was cheap or many of the companies could never have survived. The greatest problem was finding information which would help to solve some of the daily problems encountered in the packing of new products and to prevent the spoilage encountered in the processing of low-acid products.

Many of you present here today probably have had the opportunity to see the food industry grow out of the pre-Food Law period to the status it has now attained. This growth was the result of the efforts of dedicated men with energy and vision who did their own research though lacking the advanced instruments and equipment used today—yet with effective results—even as Nicholas Appert did in the early 1800.'s.

During these initial stages of the development of the food industry, there were few individuals who were trained to enter this field. Food Technology, still in its infancy, was not even recognized by its present name. Courses in colleges dealt only occasionally with the applications of science to foods, and these courses were found in many different departments.

PIONEERING A NEW ERA OF HIGHER EDUCATION

The many suggestions made to me by my major professors in college plus my early association with the food industry further stimulated an interest I already had in this field. Years later, when I was offered the opportunity to come to Oregon State College and develop this type of education, no urging was necessary.

On entering college work, I was faced from the very beginning with conflicts of departmental interest. Certain of the departments who were including some food work in their lecture courses did not feel they should give these up to us. The school of agriculture demanded that we incorporate certain subjects pertaining to livestock and related agriculture in our freshman and sophomore years. These were some of the obstacles to systematizing the teaching of food technology. With constant striving, however, we finally won out, eliminated these peripheral courses, and substituted more fundamental science studies in our four-year curriculum.

We felt we had made a major step in the early 1920's in the undergraduate training of men in food technology. We realized that this was only the beginning, but we were confident that future broadening would result. Early in our career, however, we were criticized by those who felt that our course of study was too heavily dominated by scientific subjects and offered little opportunity for cultural study. As rapidly as possible we instituted changes, but the need for curriculum analysis seemed never ending in a field where such rapid changes were taking place.

A better understanding of the type of training that we felt was desired can best be given by briefly reviewing the history of our present educational system. American colleges were originally English in character. Tutors were the rule and formal discipline was emphasized rather than professional scholarship. Shortly after 1750 the trend in America was toward scientific and practical studies. The revolutionary war stimulated colleges that were secular in administration, gave free instruction, and prepared their graduates for service to the state. This was a reflection of French educational views.

By 1800 the professor had become a specialist rather than a tutor. The stimulating atmosphere of Germany attracted many scientists and scholars to study there, but before long graduate centers were organized in the United States. The elective system and coeducation followed, and the Morrill Act of 1862 stimulated the development of state colleges and universities

* The Nicholas Appert Award Address, presented by the Appert Medalist for 1960, Professor Ernest H. Wiegand, at the Twentieth Annual Meeting of the Institute of Food Technologists, San Francisco, May 18, 1960.

and, more especially, schools of agriculture. All of these developments were in the democratic tradition.

The democratic tradition influenced American higher education in many ways. It removed the restrictions to acquiring an education, and the principle of state support put higher education within the reach of the masses. One of the issues in higher education which developed early was that between the tradition of culture and vocational or professional emphasis. Originally, colleges were for the select few, the economically and intellectually gifted, and offered vocational education, chiefly for the learned professions, with a cultural content. For the most part, college students of that early day were privately prepared for college.

The public school system led to college attendance by large numbers of students neither interested in nor qualified for the "learned" professions. As need for technicians in industry, business, or agriculture grew, it was appropriate to the American tradition, that they be trained in tax-supported schools rather than in private technical schools. New or growing professions increased the needs. "Apprenticeship," a time-honored method of preparing youth for their vocations, gave way to private training institutions which soon developed into organized institutes and colleges. The small sequence of prescribed courses that characterized these colleges in the beginning curricula yielded to little better than a haphazard collection of "offerings." Baccalaureate degrees could be obtained with but few social, philosophical, or literary studies represented in the credit requirements for such degrees.

A UNIVERSITY MUST DEAL WITH ISSUES VITAL TO THE TIMES

The one principle that has emerged throughout the history of higher education, one that might well be remembered by any group planning a curriculum that is to be of importance to mankind, like food technology, is that the university flourishes when it deals with questions and issues vital to the society of the times. That the university must serve the public good is a truism as old as the university itself. When the university loses itself in five points of research that are of interest only to the school and slights investigations leading to the solution or alleviation of problems for the good of people, then it may expect to experience decline.

Another principle emerging from this period is that the body of knowledge to be studied in the university cannot become static. There must be a constant process of revision and change to add new knowledge. Unless this is done, the university faculty, the university itself, rapidly loses its perspective and becomes inadequate. This has been especially true in scientific fields in the past and it is more true today than ever.

The haphazard training of which I have spoken, a training resulting in the graduation of students with no common ground of discourse, with only a few facts and devices rather than basic understandings, led to bitter attacks by educational leaders of vision. Among those leading the crusade for fundamental educational concepts during the past decade has been Robert Hutchins, former president of the University of Chicago. Without necessarily accepting his extreme anti-vocationalism, we can commend his emphasis on teaching students to think and to deal with ideas. Hutchins' remarks indicate that the trend of engineering or technological education is now away from vocational courses, and towards increased emphasis on physics, chemistry and mathematics, thus suggesting that the feeling is growing that the best practical education is the most theoretical one. Modern educational leaders do not, however, preclude the educational value of experience. They realize that to get experience in a specific field it is necessary to engage oneself in that activity. Thus, in professional education, we are likely to see the principle of internship which has worked so well in medicine, incorporated.

THE UNIVERSITY WILL NOT SEEK TO TRAIN MERE PRACTITIONERS

James Rowland Angell writing in *American Education* stresses the fact that the period in which we are living and especially the more recent portion of it, has witnessed such a pushing forward of the frontiers of scientific knowledge, and such a revolution in the mastery of nature, as no previous epoch has remotely approached. This scientific surge has largely been engendered by discoveries following upon the process of intensive specialization. It has made the specialist himself a commanding figure in our scientific and intellectual world. An immediate result of this tendency to specialize has been the departmentalization of educational procedure. Though specialization is justified in many respects and will go steadily forward despite remonstrances, there is a crying need for a fresh synthesis of knowledge in the world of affairs, quite as much as in the world of theory.

Probably many of you will recall the survey reported in the 1941 *Proceedings of the Institute of Food Technologists* by Larry V. Burton, former editor of *Food Industries*, wherein he stated that manufacturers were clamoring for men of culture, those who could get along with their fellow men, who were at ease with the president of the organization and who were, additionally, "regular fellows" with those whose occupations were rated far down the scale of skills in the plant.

This was not an unusual point of view, as was brought out by Paul Hanus in an article in the *Journal of Higher Education* in 1940. Quoting him, "To my mind, general culture, so far as it can be secured through instruction, means specialized knowledge and skill in some one field of the resources of our civilization, literature, history, government, economics, science, or art, and sufficient knowledge in the fields of those same resources, as will tend to develop an appreciation of their significance to society. It matters greatly if his specialty does not lie in the field of social studies, or in the humanities, that his choice of work outside his specialty includes studies from those fields. From the humanities just because they are

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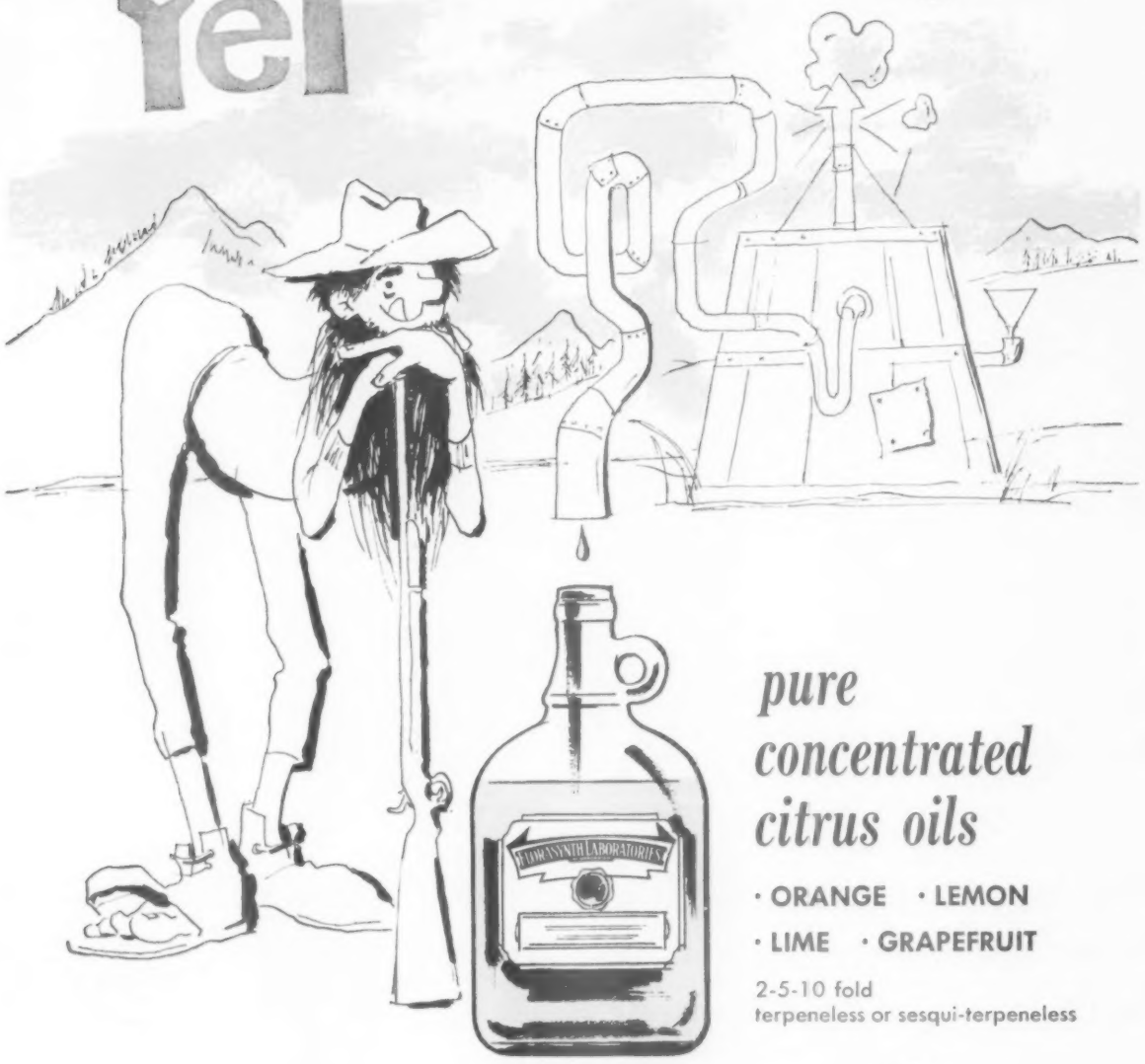

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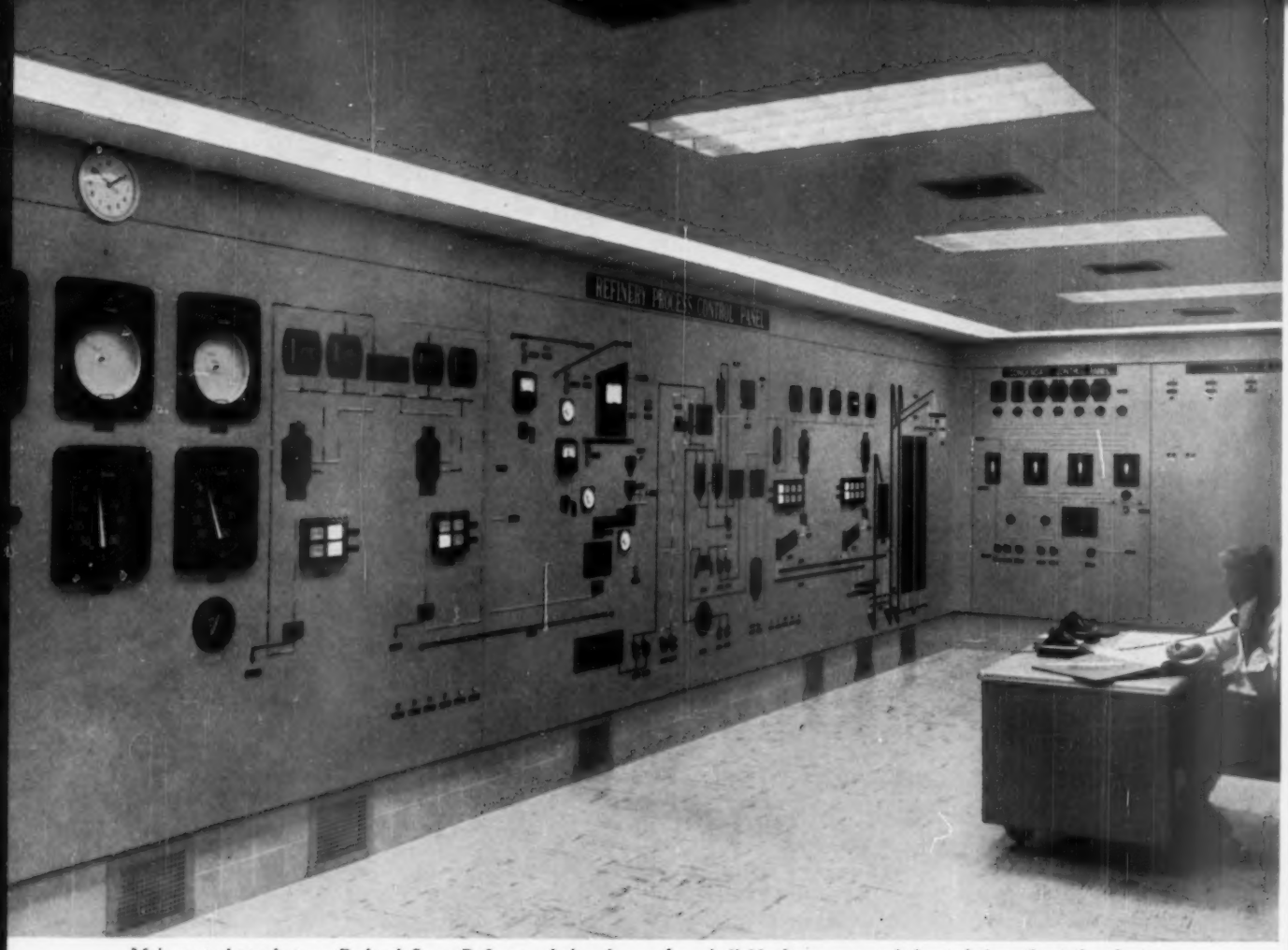

Main control panel at new Redpath Sugar Refinery—designed and built by The Foxboro Company, Limited. Process data from individual process panels located throughout the plant are also recorded here.

Foxboro control selected for new Toronto sugar refinery

"We liked Foxboro's policy of 'intelligent' automation"
Canada and Dominion reports

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humanizing and from the social studies because of their value in his equipment as a citizen, no matter what his future career may be, be it teaching Greek, or working in public service, or in business, or industry. The university will not seek to train mere practitioners. It will seek to provide training for leadership in the future career of the student. No part of the work of the university is more important than training for citizenship. Our democracy requires leadership and the enlightened cooperation of all citizens. The university will try to do all it can to prepare its graduates for such leadership and cooperation. Social studies must have a prominent place in the scheme of studies, a place second to none. The serious pursuit of these studies, well planned, with good teaching must include as much contact with actual government especially local government, and as much study of industrial concerns as possible. Not merely occasional visits to the city hall, or commercial houses, but so far as possible, a real study of how local and state office holders do or do not, adequately and conscientiously discharge their responsibilities, with a similar real study of the policies and procedures of industrial plants and commercial enterprises."

As a result of these and other criticisms, there has been a tendency to stem the disintegration and individualization of studies in the elective system by introduction of some common courses in contemporary civilization, the humanities, and the sciences for orientation or synthesis. Here again care must be taken lest the student fails to get a well rounded picture of the world about him. It has been true that students, in the whole of the educational process, have failed to be brought up to date. In history or literature, for example, the whole of their exposure may have been to past persons and events, with no opportunity to learn of the work and significance of the present. The implications of the tendency to correct these failures would seem to be that the modern university, or college, is recognizing a responsibility for the education of its students, in the sense that each, at some point in his educational career, must be brought to a realization of the bearing of his own special field of study, whether it be law, medicine, engineering, or teaching, on the social and intellectual life of his day. The modern university must dig deep into contemporary life, thereby, not only transmitting culture but also recreating it in accordance with the demands of a changing civilization.

From I. J. Kandel on the "Problems of the University" reported in *The Educational Forum*, 1938, we have this line of thought: "If danger of specialization is genuine, the distinction upon which the universities have prided themselves between academic and vocational or professional, between pure science and applied science, between speculative and practical, must break down and the two aspects be conducted side by side, each invigorating the other.

The first function of the university, is to put the student in the way of acquiring a knowledge and a mastery of his own field of study. It must go beyond this and train the student not merely to acquire knowledge, but to understand its practical suggestiveness for life, and to appreciate the directions in which further advancement is needed. Too often university professors have forgotten that the printing press has been invented, and spend their time in the mere repetition of what may be found in books. The interpretation of that knowledge which is established, the appreciation of the unexplored; these should be the chief concern of the instructor in the class room."

It would be unfortunate for me to discuss with this group the details of setting up a food technology curriculum, pointing out all the courses which I feel would be essential in the light of the criticisms which I have pointed out other educators have indicated regarding curriculums in university training. In view of the fact that the Institute of Food Technologists through its committee on education is now engaged in developing a program to improve the curricula in food technology, I would not wish to inject my views on the details of such a program. However, I do feel in the light of the criticisms which have been made that careful consideration of these points be taken in preparing a program which colleges and universities would accept.

It is agreed that a broad background as conceived by some would require time in any professional course. Four years seems inadequate for cultural training plus the fundamental background the food technologist must have. The trend in some institutions toward a five year curriculum in some professional courses is a worthy one. A preliminary study of the fundamental sciences for a bachelor's degree for the food technologist before major work in this field, would broaden his training and give him an even better background.

As educators, we may often lose sight of the student's problem, however. His primary interest may be the field of food technology, but personal circumstances prevent him from spending more than four years in college. This may not apply to all students, but the percentage who are unable to prolong their education beyond four years, is many times greater than those who can. Bearing this in mind, it seems essential that we try to pattern our program to better fit the majority who have limited time to complete their education, thus providing trained manpower for our industries.

For qualified students the program can be extended even beyond the limits now set. In many cases these students are doing their work on scholarships or fellowships which makes it possible for them to prolong their education and obtain higher degrees.

THE IMAGE OF A FOOD TECHNOLOGIST

The discussion thus far has been most general. A little more detail on educating the food technologist may clarify the ideas I wish to emphasize. First, let us say the food technologist must be trained in the basic scientific principles upon which food technology is founded. He should be well versed in chemistry, physics, mathematics, bacteriology, and engineering. Manufacturers of food products are overwhelming in

their stress on the importance of these foundations. This is demonstrated in the model curriculum designed in 1958 at the Allerton House Conference sponsored by your committee on education. Also, let us be sure that the food technologist has had enough bacteriology to deal with the problems of sanitation and the actions of microorganisms in food. He must be a chemist to understand the many reactions that take place during the processes applied to food in manufacture. His engineering background should be adequate for him to understand preservation by heat, refrigeration, dehydration, and radiation. His training in this field need not be as extensive as that of the student majoring in engineering; yet he should be able to recognize certain signs and symptoms indicative of operational irregularities in the plant. In addition, the food technologist should have a knowledge of the basic principles of human nutrition and of food preservation as well as the underlying basic problems concerning food manufacture.

Even the four year student should have some training in research and development methodology and its literature since a large part of his work will relate to new developments. If he is employed to develop new products or even improve those then being manufactured, he will need such training. Usually such work is presented in the senior year, after a thorough course study in the fundamental sciences.

The demand for food technologists is for those with enquiring minds, imagination, practical ideas, and limitless persistence. The importance of a training in research, is superseded only by his training in the humanities and in the fundamental principles of science.

To round out the training of the food technologist, he should be subjected to courses in accounting in order that he may be able to interpret the profit and loss record accurately, to courses in business organization, management and law so that he will be conversant at least with the problems of the manager—and enough instruction in salesmanship to understand the problems of placing products before the public.

Another point I wish to stress is the invaluable education a student would receive by being properly placed in a food plant where management would see his potentials and become interested in his education. It is our belief that not less than two summers should be spent in this manner and preferably more. Prior arrangements should be made with management in such a plant so that the student may move about and participate in the work of different departments even assuming a share of the responsibility, thus enabling him to become acquainted with the tasks and the obligations of different positions.

Reports upon his work from management should include his aptitude, his ability to get along with others, and his efficiency. His own evaluation of the work he did should be reported, including his reactions as to the efficiency of the plant set-up, labor problems, sanitary procedures, and general operating conditions. His suggestions for possible plant improvement should be included.

To encourage the student and to assist in his professional development, plant seminars have been suggested. I think this well worthy of consideration. It will stimulate the student to develop ideas, to see the problems—and to make a real effort to find solutions even though not particularly encouraged to do so by management directly over him. Too often when the student has left the plant, the situations he encountered have lost their importance to him, and he does not follow through to a satisfactory conclusion in his own thinking.

Students should be encouraged to attend open meetings of industry and conventions or even schools held by processors. In this way, they gain valuable information and at the same time, meet and learn to know the men important in the field which they may wish to follow.

There are among our students those for whom we must assume some responsibility. They are the students who do not have the financial background to continue through a four year course of study; yet they have an interest and aptitude for this field of work. For these, training in one of the operational branches of vocational food technology would be desirable. These men could take courses dealing with the applications of chemistry, physics, bacteriology, and engineering. The applications of these sciences are essential for him to understand if he is to converse intelligently with thoroughly trained men directing the over-all work of the plant. Vocational study would cover a two year period, and the student completing it should receive a certificate. Here, the broad cultural training would not apply.

IN SUMMARY

To summarize, the food technologist is destined to assume an increasing role in our ever growing food industry. With the rapidly changing pattern of this industry trained graduates from our universities will be in great demand. It, however, behooves us to see that our graduates are properly trained in the fundamental sciences and engineering that underlie food technology. We must recognize and accept the fact that industry will be critical and will demand that the men they employ are able to get along with their fellow man, assume their obligations as citizens, express their conclusions and convictions clearly, and have a faculty for diplomacy, tolerance and consideration. Technical training is not alone sufficient for the business or professional man.

The training of the food technologist will eventually culminate in a five year course. However, the present four year course, if properly developed for the student who does not wish to become a specialist, is adequate to meet his needs, so that he may be able to assume a position in one of several phases of the food manufacturing industry. The number of men able to undertake specialized training will be limited, but it is the privilege and responsibility of the university or college to see that those who can contribute most are properly prepared.

Chemical Composition of Raw, Precooked, and Canned Tuna. I.

Core Sampling Methods[a]

Clarence J. Carlson, Claude E. Thurston, and Maurice E. Stansby

Technological Station and Technological Laboratory,[b] Bureau of Commercial Fisheries, Terminal Island, California, and Seattle, Washington

(Manuscript received May 18, 1960)

In studying the differences in composition of tuna at various processing stages, the problem arose as to how representative samples could be obtained without mutilating the entire fish and rendering it unsuitable for canning. In commercial tuna canning, the raw, butchered fish is given a precook, and the edible light meat is separated from skin, bone, and dark meat. The light meat, in the form of tuna loins, is then sliced, placed in cans with certain additives, and heat processed. It is sometimes desirable to obtain a representative sample from the original, raw fish of those portions which will eventually end up in the can for analyses of various components such as general freshness, degree of rancidity, or proximate composition.

Core sampling provides a means of taking from the raw tuna small portions at various locations and by selection of the sampling points, the sample can be taken to avoid bone or dark meat so as to be typical of the light meat which will end up in the can. A series of experiments was therefore carried out to determine whether a simple core sampler *(2)* would give sufficiently homogeneous samples to be used for accurately carrying out several subsequent analyses including proximate composition, sodium and potassium, total volatile base (a freshness test), and tests on the oil by the thiobarbituric acid method for rancidity and for free fatty acid. It was realized that variation of some of these constituents from one part of the fish to another might be much greater than that for others. Thus, a simple coring procedure might work well for some analyses and be almost valueless for others. The primary purpose of this work was to evaluate the possibility of using such a coring method for sampling for these various determinations. Some incidental work was done on minor modifications in the way in which the core sample was taken.

No attempt whatever was made in this work to determine the size of sample needed to be representative of a given lot (e.g., in a well on a tuna vessel).

EXPERIMENTAL METHODS

The distinctive structure of a tuna-like fish is shown in cross-section in Figure 1. The dark meat is found near the center and is surrounded by the light meat. Two sets of bones extend from the spine at right angles to one another, dividing the flesh into 4 parts called "loins." This structure required a minimum number of 4 cores for a representative sample (Figure 2). For a large fish, more samples could be removed but 6 would probably be adequate. Many sizes of commercial plug-cutters were available, but only two were selected. They were ¾ and 1 inch in diameter, by 2 and 1½ inches in length, respectively. Sizes beyond these limits were considered too small to be readily handled or so large as to damage the fish for further use.

Collection and storage of specimens. This investigation was limited to the small size fish since they presented the greatest difficulty in obtaining representative samples. Three series of fish were collected ranging from 7 to 12 lb in weight with an average of about 9 lb. They were purchased in January, April, and August 1959, and represented different species as well as different areas of capture and methods of preservation on board the vessel. There was no intention, however, to consider

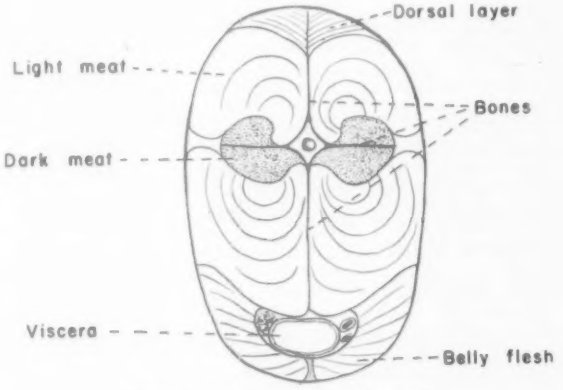
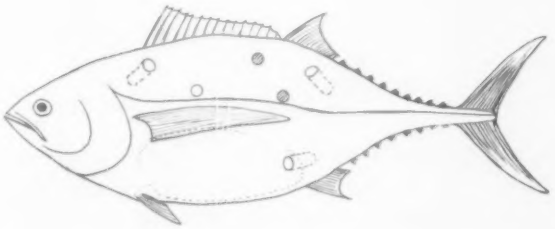


Figure 1. Cross section of a tuna.



5th and 6th cores

Figure 2. Lateral view of a tuna—position of cores.

[a] Presented at the Twentieth Annual Meeting of the Institute of Food Technologists, San Francisco, California, May 16, 1960.

[b] A laboratory of the Division of Industrial Research and Services, U. S. Fish and Wildlife Service, Department of the Interior.

TABLE 1

Effect of number of cores per fish on reproducibility of various analyses of tuna

Sample number	No of fish per sample	Sampling method	No of cores from each fish	Moisture %	Oil %	Protein %	Ash %	Sodium Mg %	Potassium Mg %	Total volatile base Mg N/100 g	Thiobarbituric acid test $E^{1\%}_{1\,cm}$
1	1	C	12	73.2	1.53	27.0	1.51	200	700	89	0.32
		E	0	73.2	1.36	27.4	1.45	280	560	90	0.20
2	1	C	12	74.0	1.33	28.2	1.41	250	390	90	0.39
		E	0	73.8	1.21	26.6	1.41	240	650	90	0.18
3	1	C	12	76.9	0.42	23.4	1.43	150	310	87	0.27
		E	0	76.7	0.61	23.3	1.37	120	330	124	0.38
4	1	C	4	74.4	0.72	24.7	1.49	130	330	17	0.69
		E	0	75.6	0.45	24.7	1.48	120	330	42	0.11
5	1	C	4	74.9	0.70	24.5	1.47	110	360	148	1.32
		E	0	74.1	0.45	25.2	1.46	150	330	158	0.16
6	1	C	4	72.8	1.75	25.6	1.54	110	310	150	1.48
		E	0	73.2	1.86	25.0	1.40	110	320	125	1.15
7	6	C	12	74.5	0.69	24.4	1.51	120	340	136	0.11
		E	0	74.6	0.72	24.2	1.44	150	310	148	0.08
8	6	C	4	74.2	1.02	24.6	1.44	120	320	154	0.12
		E	0	73.5	1.02	25.1	1.42	150	270	151	0.15
Average		C		74.4	0.96	25.0	1.46	139	366	121	0.43
		E		74.2	0.93	25.1	1.43	163	355	127	0.24

C = Core sample.
E = Remaining edible flesh.

in this preliminary study the influence of season, species, area of capture, or method of preservation on the composition of the fish. The specimens were frozen, if not received in that condition, and held at −10° F until processed.

Preparation of samples. The fish were stored overnight in a refrigerator in order to bring the temperature to about 25° F. An attempt was made to remove the samples when the flesh temperature was between 26° and 30° F, the operating range employed in the canneries. The plug cutter was used to remove cores of the desired size and number from the fish after which the remaining light meat was removed. If the plug was not withdrawn by the cutter, a wire spoon was used to cut it free at the bottom and remove it from the fish. After removal, samples were kept under refrigeration in friction-top cans while successive specimens were processed. As each sample was taken from the fish it was freed from any bones, skin, or viscera removed with it. The 4 composite samples were each ground in a food chopper until homogeneous in consistency; portions were then sealed at atmospheric pressure in ½-lb cans, frozen, and stored at −10° F.

Methods of analysis. Each sample was analyzed for the groups of chemical constituents: (1) proximate composition, including moisture, oil, protein, and ash; 2) the mineral constituents sodium and potassium; and (3) constituents reflecting the quality or state of preservation, namely, total volatile base (TVB), the thiobarbituric acid test (TBA) for rancidity, and free fatty acid in the oil (FFA) (not included in series 1).

Moisture, oil, protein, and ash were determined by using standard procedures of the Association of Official Agricultural Chemists (AOAC) for fish and other marine products *(1)*. Sodium and potassium values were obtained with a flame photometer using solutions prepared from the ash residues by extraction with dilute nitric acid, and diluting to appropriate volume *(5)*. The total volatile base was determined by the distillation procedure of Stansby, Harrison, Dassow, and Sater *(4)*. The thiobarbituric acid test was made according to the modified procedure of Yu and Sinnhuber *(6)*. The values are given in per cent absorption per cm of solution ($E^{1\%}_{1cm}$). Free fatty acid values were determined by the method of the American Oil Chemists' Society (AOCS *(3)*.

RESULTS AND DISCUSSION

An experiment was carried out to determine the number of cores per fish which would be required to obtain sufficiently representative samples. In this experiment in some samples four 1-inch cores were taken from one side of a fish; in other cases six 1-inch cores were taken from each side of the fish (Figure 2). Thus a comparison was made of results in which four vs twelve 1-inch cores were used per fish. In this first experiment, in some cases, the cores from individual fish were analyzed separately (i.e., composites of cores from one fish) and in other instances composites of cores from 6 fish were analyzed. Results of this experiment (Table 1) indicate that for most determinations sufficiently representative samples are obtained when only 4 cores per fish were taken and with little or no improvement in precision resulting from the use of 12-core samples. A comparison of the results on the edible flesh minus the cores (E) which may be considered the "correct value" with those on the core sample (C) gives a measure of the reproducibility. An exception is noted in the case of the thiobarituric acid test for rancidity where much more reproducible results were obtained for the 12-core samples. Even here the reproducibility was not good.

A second experiment was carried out employing 4 cores per fish throughout but using in some cases 1-inch cores, inother cases ¾-inch cores. In this series an additional analysis, free fatty acid of the oil, was included. The different sized cores were taken from alternate sides of the fish, the ¾-inch core from the right side and the 1-inch core from the left side. In this experiment it was assumed that there was no difference in composition of the left or right side of the fish. This was probably the case for most of the analyses. Perhaps the salt content may have varied since the salt content in tuna frozen in brine depends partially on their position at time of freezing with respect to other fish (e.g., where 2 fish freeze close together, salt penetration will be greater on the sides exposed to the brine than in the sides touching each other).

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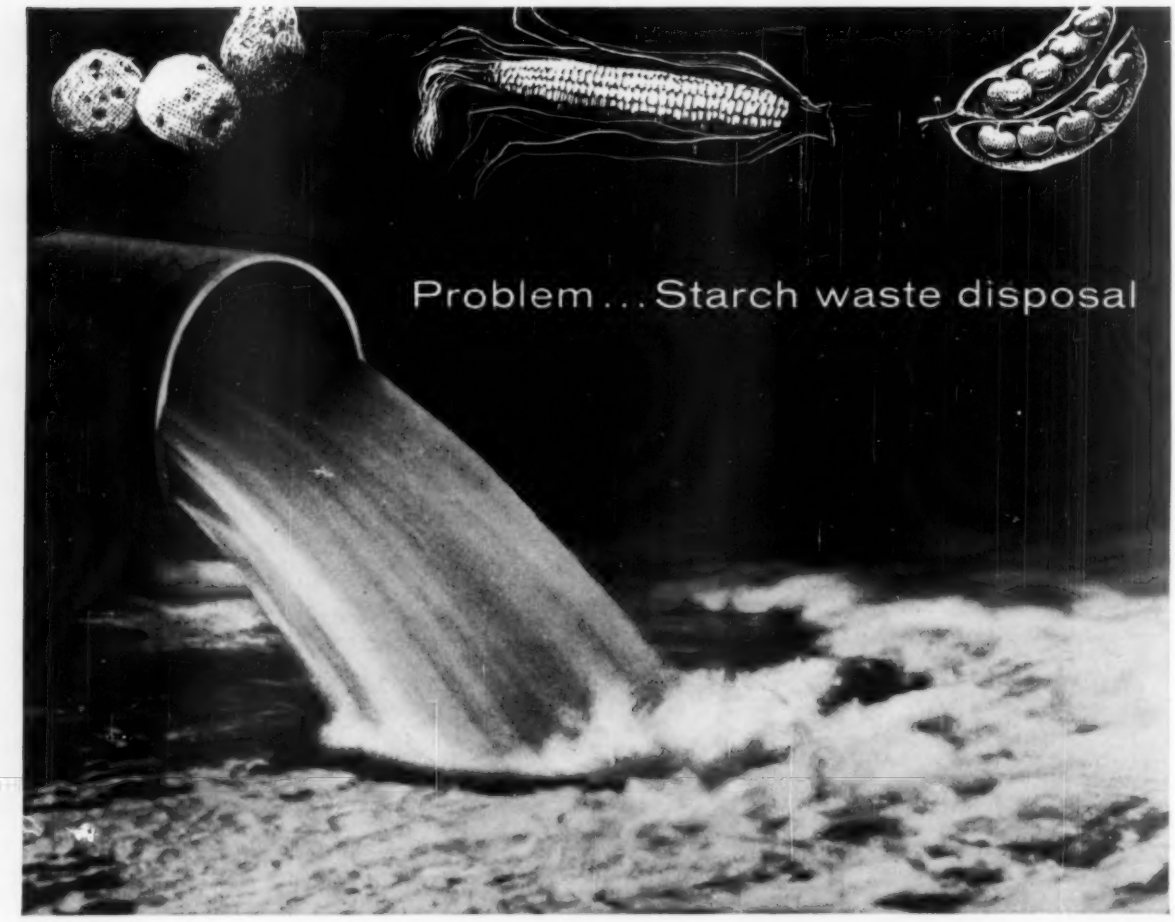

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TABLE 2

Effect of core size (¾" or 1") on reproducibility of various analyses of tuna

Sample No[1]	Part used	Moisture %		Oil %		Protein %		Ash %		Sodium Mg %		Potassium Mg %		TVB Mg N/100 g		TBA E–%/cm		FFA as oleic %	
		¾"	1"	¾"	1"	¾"	1"	¾"	1"	¾"	1"	¾"	1"	¾"	1"	¾"	1"	¾"	1"
1	C	70.3	70.1	1.99	1.76	26.4	27.1	2.46	2.26	568	509	320	358	.....	.....	0.40	0.38	12.3	6.1
	E	70.0	69.7	2.36	2.32	26.4	26.4	2.55	2.58	642	677	354	340	.....	.....	0.33	0.24	10.0	11.4
2	C	69.8	69.5	1.88	2.22	27.0	27.5	2.16	2.20	471	482	373	372	14.0	11.4	0.36	0.28	5.5	5.0
	E	69.2	69.0	2.64	3.16	26.7	26.6	2.58	2.48	643	611	349	358	10.1	14.4	0.33	0.44	5.8	6.4
3	C	69.8	69.4	2.05	2.48	26.7	26.5	2.26	2.52	540	642	350	362	5.0	9.0	0.44	0.35	5.5	5.2
	E	69.5	69.2	2.45	2.80	26.6	26.7	2.69	2.69	685	673	342	356	13.8	12.0	0.33	0.36	4.5	6.5
4	C	69.3	69.3	2.58	3.60	27.2	26.7	1.96	2.10	463	478	346	350	10.5	11.9	0.31	0.18	6.4	9.2
	E	69.1	68.8	3.32	3.79	26.4	26.5	2.29	2.28	548	551	348	361	19.6	10.7	0.14	0.18	9.6	9.9
Average	C	69.8	69.6	2.13	2.52	26.8	27.0	2.21	2.27	515	528	347	358	9.8	10.8	0.38	0.31	7.4	6.4
	E	69.5	69.2	2.70	3.02	26.5	26.5	2.50	2.50	630	626	348	354	11.2	12.4	0.28	0.30	7.5	8.5

[1] Each sample represents 6 fish.
C = Core.
E = Remaining edible flesh.

TABLE 3

Reproducibility of various analyses using four ¾-inch cores per fish

Analysis		Reproducibility[1] Expressed as deviation from true value[2]				
Component	Units	Sample No 1	Sample No 2	Sample No 3	Sample No 4	Average
		%	%	%	%	%
Moisture	%	0.6	0.9	1.6	0.7	1.0
Protein	%	0.4	0.8	3.1	0.8	1.3
Oil	%	3.8	24	38	27	23
Ash	%	0.8	5.7	11.0	9.7	6.8
Sodium	Mg %	7.3	11.6	10.9	13.9	10.9
Potassium	Mg %	3.7	1.9	3.7	4.2	3.4
Total volatile base	Mg N/100 g	3.3	10.4	1.8	15.9	7.8
Thiobarbituric acid	$E\ \frac{1\%}{1\ cm}$	176	432	217	205	258
Free fatty acid	% as oleic	5.2	0	15.8	6.0	6.8

[1] Each replicate sample represents 4 cores per fish from 6 different fish.
[2] The true value is taken as the value obtained on the composited white meat after the removal of the cores.

The results obtained with the two different sizes of cores (Table 2) indicate that except for the thiobarbituric acid test for rancidity, about equally good results are obtained with the ¾-inch or 1-inch sized cores.

Results in Table 3 indicate that the use of a 4-core sample gives reproducible results for moisture and protein analyses, reasonably reproducible results for potassium, ash, free fatty acid, total volatile base, and sodium, fair to poor reproducibility for oil content, and very poor reproducibility for thiobarbituric acid determination. These degrees of reproducibility are doubtlessly correlated with the variability of the content of these components from one part of a fish to another, and they are in line with the anticipated variability. Thus the TBA values are dependent both upon chance occurrence at the point of coring of layers of oil and of oil which has been exposed to air or is otherwise susceptible to oxidation. Oil is the component of fish which shows the greatest variability from one part of the fish to another. We would thus expect TBA values as well as oil content to vary the greatest for core samples taken at different points in the fish. Protein and moisture content would be expected to vary from one part of the fish to another the least of any of the components measured and the reproducibility of protein and moisture is reasonably high.

CONCLUSIONS

Use of four ¾-inch cores per fish (taken as shown in Figure 2) gives results which are homogeneous with respect to protein and moisture content and probably are reasonably homogeneous with respect to most other components not associated with the oil of tuna. Fair reproducibility can be obtained using these 4-core per fish samples when determining the oil content, although greater reproducibility can be obtained by using 12 or more cores per fish. The core sampling method is not recommended for use in preparing samples for rancidity tests such as the TBA method.

Acknowledgment

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Effect of Borates and Other Inhibitors on Enzymatic Browning in Apple Tissue. II.

Mechanism[a]

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(Manuscript received April 18, 1960)

BORATES HAVE BEEN SHOWN *(1)* to act as inhibitors of enzymatic browning of apple slices. It was stated that they probably functioned by forming a complex with polyphenols at the di-OH grouping which is the site of the enzyme-catalyzed oxidation causing the darkening.

The reaction of boric acid with polyhydroxy and related compounds is well known. The ability of mannitol to render boric acid more acidic forms the basis of an analytical determination for boric acid *(6)*. The principal requirement for reaction with borate is that the hydroxyl groups must be adjacent and *cis*. Compounds with more than two hydroxyl groups react more strongly, and the intensity of the reaction increases with increasing number of adjacent hydroxyl groups; i.e., glycerol, erythritol, arabitol, and mannitol, successively *(6)*. It has been shown that aromatic ortho-polyhydroxy compounds (catechol, pyrogallol, etc.) react strongly with boric acid *(2)*.

The following changes have been noted in the nature of the polyhydroxy compound after reacting with borate; increased acidity, change in optical rotation and light absorption, and decreased oxidation of mono- and disaccharides by alkaline copper solution *(3)*.

This last-mentioned effect, decreased oxidation of the substrates, is the focal point of this paper. Studies were conducted to elucidate the mechanism by which borates inhibit enzymatic browning of apple.

MATERIAL AND METHODS

Preparation of polyphenol extract. One kg of apple slices was steam blanched for 7 min to inactivate the phenolyoxidase enzymes. The slices were cooled in tap water and blended with 300 ml ice water for 2 min in a Waring blender. The puree was filtered through cheesecloth and 2 volumes cold (−40° F) acetone added to the liquid to precipitate proteinaceous matter. The precipitate was removed by filtration and the filtrate heated to drive off the acetone. The cloudy suspension was cooled and filtered. Neutral lead acetate was added to precipitate lead polyphenolate. This was separated and taken up in M sulfuric acid. The lead sulfate precipitate was removed by filtration, the pH of the filtrate was adjusted to 5.0 with N NaOH and it was then stored in the refrigerator.

Enzyme preparation. Five mg of a commercial phenol oxidase preparation (K and K Laboratories, Long Island, N. Y.; 300 units/mg) were dissolved in 25 ml distilled water.

Absorption measurements. The General Electric Automatic Recording Spectrophotometer was used to obtain the absorption spectra of oxidized catechol and oxidized polyphenol extract. All other optical density determinations were made with the Coleman Universal Spectrophotometer Model 14 at 430 mmc.

General assay procedure. As a result of experimental work to be presented, the following procedure was developed for determination of color formation in reaction mixtures: To 10 ml of pH 7.0 citrate-phosphate buffer, add either one ml of 0.005 M catechol solution or 3 ml of the polyphenol extract. Add one ml of the enzyme solution and shake. Let the reaction proceed for 30 min at 25° C and measure the optical density at 430 mmc.

RESULTS AND DISCUSSION

Development of assay procedure

Absorption spectra. Both the oxidized catechol and oxidized polyphenol extract showed an absorption peak at 430 mmc. This agrees fairly well with the wave length of maximum absorption reported previously *(5)*. Therefore, it was decided to use this wave length in measuring the extent of browning.

Enzyme concentration. A series of enzyme solutions varying in concentration from 0 to 60 units/ml was prepared. One ml of each concentration was added to a mixture of 3 ml polyphenol extract and 6 ml buffer. The optical density was read after 1 hr. From the data plotted in Figure 1 it was concluded that the enzyme was not limiting when solutions of 48 to 60 units/ml were added to the reaction. Therefore, it was decided to use 1 ml of an enzyme concentration of 60 units/ml.

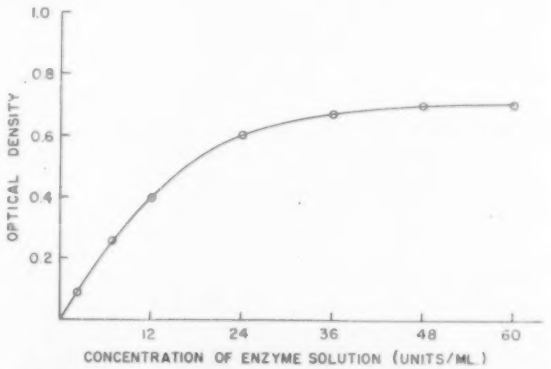

Figure 1. The effect of phenol oxidase concentration on the oxidation of polyphenolic extract.

[a] Presented before the Nineteenth Annual Meeting of the Institute of Food Technologists, Philadelphia, Pa., May 18, 1959.

[b] Submitted in partial fulfillment of the requirements for the Ph.D. degree in Food Technology, University of Illinois, Urbana.

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Buffer pH. Citrate-phosphate buffers were prepared to cover the range pH 3 to 8 in one-unit steps; glycine buffers were adjusted for pH 9 and 10. To 10 ml of each buffer solution was added 1 ml of 0.005 M catechol followed by the addition of 1 ml enzyme solution. In another series of buffer solutions 3 ml polyphenol extract was added in place of the catechol. The reaction time was 1 hr. The results, Figure 2, showed this commercial enzyme preparation to have a maximum activity at pH 7 in catalyzing the oxidation of both catechol and the phenol extract.

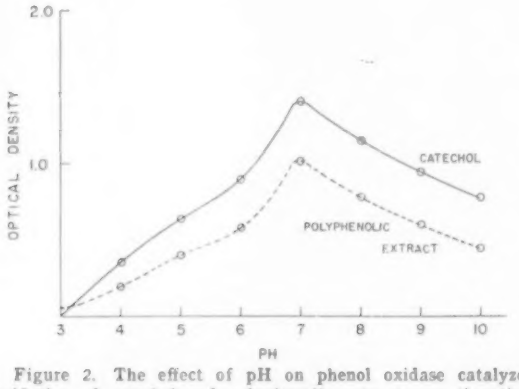

Figure 2. The effect of pH on phenol oxidase catalyzed oxidation of catechol and polyphenolic extract; reaction time sixty minutes.

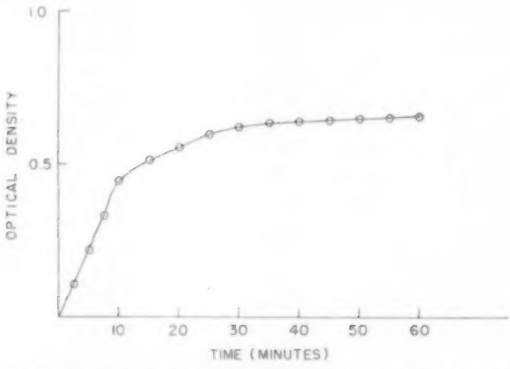

Figure 3. The phenol oxidase catalyzed oxidation of catechol as a function of time.

Reaction time. The same general procedure was followed with both catechol and polyphenol extract, except that optical density readings were taken at 5 min intervals for 1 hr. Results for catechol are shown in Figure 3. There was a sharp increase in optical density to 10 min, followed by a more gradual increase to 25 min, after which the color remained essentially constant. Similiar results were obtained with extract. A reaction time of 30 min was used in future investigations.

Oxidation of catechol in the presence of borate

Effect of pH. A pH series of reaction tubes containing buffer, catechol and enzyme was prepared as described above under Buffer pH. A duplicate series was also prepared except that 1.0 ml M sodium tetraborate (STB) was added before the enzyme. The optical density at each pH is shown plotted in Figure 4. For each series, the OD varied with pH, showing a maximum point at pH 7. At pH levels of 5 and above the reactions containing STB showed lower optical densities, i.e. less browning, than did the control oxidations. Moreover, the protection against browning afforded by the STB increased as the reaction was made more alkaline. These results are in agreement with the findings of other investigators working with various polyhydric compounds *(4)*.

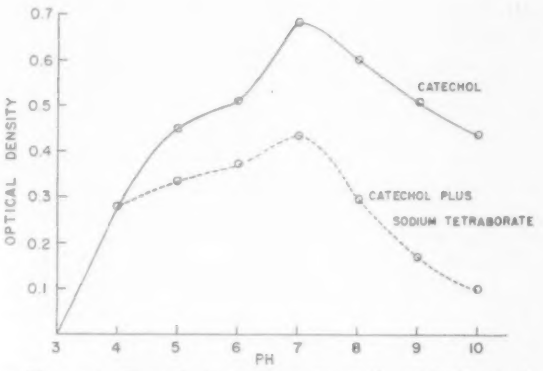

Figure 4. The effect of pH on the sodium tetraborate inhibition of phenol oxidase catalyzed oxidation of catechol; reaction time thirty minutes.

However, in our work with apple slices it was observed that the activity of STB as an inhibitor of enzymatic browning did not depend on the pH of the solution. The explanation for this inconsisteney may be that other constituents of the apple tissue affected the reaction. For example, the natural buffer system of the apple may have counteracted the buffer of the treatment solution. This concept will be amplified below.

Effect of borate concentration. Solutions of STB and of boric acid were prepared at concentrations of 1.0, 0.1, 0.01 and 0.001 M. For each solution 1 ml was added to 8 ml buffer and 1 ml of 0.005 M catechol solution and the browning development was determined by optical density measurements.

Data in Table 1 show close agreement between STB and boric acid at each concentration. In both cases the color formation decreased appreciably as the concentration was increased, especially in going from 0.0001 M to 0.001 M. This indicated that the borates are reacting with the catechol and that the amount of

TABLE 1

The effect of STB and boric acid concentration on the enzymatic oxidation of catechol at pH 7

Concentration molarity	Optical density with STB	Optical density with boric acid
0.00	0.94	0.96
0.0001	0.85	0.86
0.001	0.62	0.61
0.01	0.50	0.51
0.1	0.45	0.45

catechol thus complexed is increased with increasing borate concentration.

Although boric acid inhibited the oxidation of catechol in this "pure" system, it did not inhibit the oxidation of natural polyphenols in apple tissue. This may have been due to a difference in pH. In the test tube the solution was buffered at pH 7 while boric acid itself in solution has a pH of 5. Since apples have a pH of 3.5, strongly buffered, the pH at the interface between apple slice and boric acid solution was between 3.5 and 5. This level was probably too low for the complexing reaction. The STB was effective on apple slices because its normal pH of 9 probably resulted in a more neutral pH at the interface.

Oxidation of purified polyphenolic extract in the presence of borate

Effect of pH. The experiment outlined above on effect of pH was repeated using our purified polyphenolic extract in place of catechol. The results, Figure 5, show the effect of pH to be very close to that

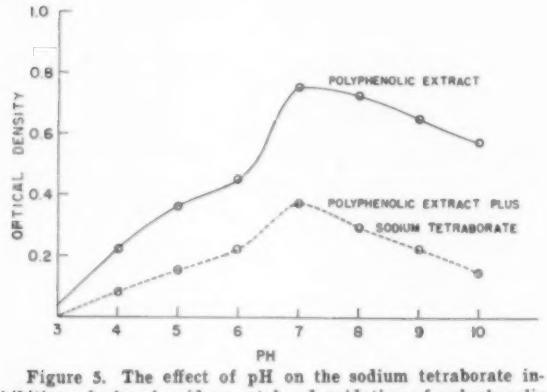

Figure 5. The effect of pH on the sodium tetraborate inhibition of phenol oxidase catalyzed oxidation of polyphenolic extract; reaction time thirty minutes.

shown in case of catechol oxidation (Figure 4). The optimum pH for this reaction was again shown to be 7. The slight difference between the findings with apple polyphenols and with catechol was that STB materially reduced browning of the extract at pH values of 4 and below. This indicated that the natural polyphenols are more reactive with STB than is catechol under such highly acid conditions.

Effect of borate concentration. The experiment on borate concentration was repeated using 3 ml of the purified polyphenol extract in place of 1 ml catechol solution. Results are presented in Table 2. Again,

TABLE 2

The effect of STB and boric acid concentration on the enzymatic oxidation of purified polyphenol extract at pH 7

Concentration molarity	Optical density with STB	Optical density with boric acid
0.00	0.88	0.86
0.0001	0.78	0.77
0.001	0.60	0.61
0.01	0.46	0.45
0.1	0.40	0.41

STB and boric acid gave almost identical results, showing that the inhibition of the enzyme catalyzed oxidation of natural polyphenols was dependent on the concentration of borate present and the greatest increase in protection was afforded in going from 0.0001 to 0.001 M borate. This indicated that borate ion inhibits browning by complexing the polyphenols which serve as precursors for browning development.

Reaction between catechol and borate

Decrease in pH. As stated above, the reaction of borate with polyhydric compounds is accompanied by an increase in acidity. Therefore, if there is a reaction between catechol and borate a lowering in pH should be detectable when the two are mixed. To test this hypothesis, 1 ml of 0.001, 0.01, 0.10 and 1.0 M boric acid solution was added to 20 ml of 0.025 M catechol. Resulting pH values were 5.20, 4.62, 4.33 and 4.20, respectively. This reduction in pH was evidence that the borate inhibits browning by complexing polyphenols present.

Non-enzymatic oxidation of catechol. It was desired to determine whether the borate inhibited only enzyme catalyzed oxidations or if it directly affected the reaction even in absence of enzyme. Four solutions containing 1 ml of 0.005 M catechol and 10 ml citrate-phosphate buffer at pH 7 were prepared. To these were added one ml STB solution in concentrations of 0.001, 0.01, 0.1 and 1.0 M. The optical densities after 24 hr at room temperature were 0.86, 0.61, 0.50 and 0.46, respectively. This decreased browning with increased borate concentration is further evidence that borate acts by affecting the polyphenols rather than the enzyme.

Ultra-violet absorption. Catechol has an absorption peak in the ultra-violet region at 280 mme wave length *(5)*. It was of interest to determine whether the absorption at this wave length was affected by borate. To a series of 4 tubes containing one ml 0.005 M catechol and 20 ml citrate-phosphate buffer at pH 7 was added one ml STB solution of 0.001, 0.01, 0.1 and 1.0 M concentrations. The optical density against a buffer-STB blank at 280 mme was 1.43, 1.15, 0.90 and 0.70, respectively. This regular decrease in optical density with increasing borate concentration was a strong indication of complex formation between borate and polyphenol.

SUMMARY AND CONCLUSIONS

The mechanism by which borates inhibit enzymatic browning of apple slices was investigated. Both catechol and a purified polyphenol extract of apple were used as substrate and a commercial phenol oxidase enzyme preparation was used as the catalyst. The reaction was allowed to proceed for 30 min at 25° C and the optical density measured at 430 mme. The point of maximum enzyme activity was at pH 7.

Borate inhibition was increased by increasing pH of the system and by increasing borate concentration. Sodium tetraborate and boric acid gave closely similar results as inhibitors as did catechol and polyphenol

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apple extract as substrate. Increasing amounts of catechol added to a boric acid solution caused successive reductions in pH. Borate inhibited oxidation of catechol in absence of enzyme. Addition of borate caused a decrease in the absorption by catechol of ultraviolet light. Based on this evidence it was concluded that borate inhibits the browning reaction by forming a complex with the substrate and thus preventing oxidation.

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Thermal Resistance of Microorganisms to Dry Heat: Design of Apparatus, Operational Problems and Preliminary Results[a, b]

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The nature of the atmosphere surrounding microorganisms during heat sterilization influences the effectiveness of the process; therefore, it is necessary to distinguish between a sterilization process where the cells are surrounded by a medium or atmosphere containing liquid water and one where the atmosphere surrounding the cells does not contain water in the liquid state. The terms "wet heat" and "dry heat" are used to describe these two sterilization processes. Wet-heat sterilization conditions exist when microorganisms are subjected directly to saturated steam in an autoclave or heated in a closed vessel, such as a can of food, that contains an appreciable amount of water. Dry-heat sterilization conditions exist when the microorganisms are exposed to superheated steam or air.

Interest in dry heat sterilization has been stimulated by the Dole aseptic canning system *(5)*, which uses superheated steam as a source of dry heat for sterilizing the containers and covers prior to aseptically canning the sterile food product.

The literature contains considerable data relative to the wet-heat resistance of microorganisms; however, there are comparatively few data regarding dry-heat resistance. Both Walter *(11)* and Perkins *(6)* discuss the use of dry heat for the sterilization of bacteriological and hospital supplies and recommend one hour at 320° F (160° C) for glassware and cutting instruments. Tanner and Dack *(10)*, studying the dry heat resistance of spores of *Cl botulinum*, reported that the time required to kill *Cl botulinum* spores varied from 15 to 60 minutes at 284° F (140° C) and from 5 to 15 minutes in the range 320° to 356° F (160° to 180° C). Collier and Townsend *(3)* reported thermal resistance data for spores of three organisms subjected to superheated steam: *B. stearothermophilus* (1518) $F_{350} = 0.708$, $z = 26°$ F; *B. polymyxa* (PSO) $F_{350} = 0.667$ and $z = 28°$ F and PA 3679 $F_{350} = 0.625$ and $z = 60°$ F. (F_{350} is the minutes at 350° F to destroy the organism under specified conditions; z is a measure of the slope of the thermal death time or thermal resistance curve.) Schmidt *(8)*, reviewing the resistance of microorganisms to dry heat, states, "In general, most of the methods used for measuring resistance to dry heat in the temperature range above 212° F have not had a very high degree of precision. . . ."

[a] Journal Paper No. 2624 of the Michigan Agricultural Experiment Station.

[b] Presented before the Nineteenth Annual Meeting of the Institute of Food Technologists, Philadelphia, Pennsylvania, May 19, 1959.

The primary purpose of this paper is to describe precision equipment developed for determining the dry-heat resistance of microorganisms. In addition, some problems encountered in dry-heat testing, the evaluation of the lag correction factor, and preliminary dry-heat resistance data for one species of microorganism to superheated steam are presented.

EXPERIMENTAL

Development considerations. Precision control and measurement of time and temperature was the principal design consideration in developing this apparatus for dry heat thermal resistance testing. An adequate temperature range, flexibility as to heating medium (superheated steam, air, saturated steam), asepsis, and the need to test replicate samples were also important considerations. Mechanical operation, convenience, and economy were given secondary consideration.

A basic problem of both dry-heat and wet-heat testing apparatus is moving the samples in and out of a controlled temperature and pressure chamber. This problem of movement and leakage was given extensive consideration. The fact that under dry-heat conditions the heating medium could not be expected to lubricate any moving parts was also considered.

A double-seal, two-step operation was developed instead of using a sliding plate *(9)* or double pistons with rings *(7)* to obtain a more positive seal and eliminate possible lubrication problems. In this design a shoulder supporting an "O" ring near the end of the sample-carrying pistons seals the exposure chamber while the samples are being heated, and a seal-bar located inside the chamber closes the sample entry ports the remainder of the time. A single, double-acting pneumatic cylinder located in the center rear of the machine operates the seal bar. Two double-acting pneumatic cylinders, one located on each side of the apparatus, move the samples from the loading position to the exposure position and, after heating, return them to the dumping or subculturing position. The sample cups are identical to those used by Pflug and Esselen *(7)* and are supported by two $\frac{1}{16}$-inch diameter stainless steel wires which are welded into and operated by a $\frac{1}{2}$-inch trip shaft located inside the sample-carrying pistons. The trip mechanism is located to the rear of the pistons and is cam operated. Leakage past the cup supporting wires is prevented by an "O" ring on the trip shaft.

Figure 1 is a photograph of the complete apparatus; Figure 2 is a cut-away view showing the exposure chamber and related parts.

The time control system was designed so that the entire operation will proceed automatically through the operational sequence when the start button is actuated.

The temperature control system for dry heat consists of a temperature recording pneumatic controller (thermocouple sensing element), a pneumatic needle valve, an electrically-operated superheater with a variable transformer input control and heaters and variable transformers for maintaining the thermoresistometer wall temperatures. A steam reservoir maintained at constant temperature by a pressure controller supplies the saturated steam to the superheater. The hot gas temperature controller modulates the flow of gas to maintain a constant temperature.

Operational problems. Two types of problems were encountered in putting this apparatus into operation: (1) controlling the temperature change of the gas as it flows through the apparatus and (2) obtaining uniform sample cup temperatures.

The temperature drop problem is a result of the low heat capacity of the gas. It was calculated that when the flow rate was 2.5 cfm, the removal of 1 BTU/min or 60 BTU/hr will reduce the temperature of superheated steam 26.1° F and air 32.3° F. When the thermoresistometer is operated so that the outlet temperature is only 5° F below the inlet temperature, the heat loss from the exposure chamber must be less than 0.2 BTU/min (12 BTU/hr) for superheated steam and less than 0.16 BTU/min (9.6 BTU/hr) for air. Therefore, the wall temperature of the exposure chamber is critical. The top, bottom, and front of the apparatus were fitted with electrical heaters to maintain the wall temperatures; thermocouples were attached to the inside of these walls so that the wall temperature could be measured and then controlled. During operation it is necessary to maintain the top, bottom, and front of the apparatus about 5° F above the exposure temperature to obtain the desired temperature control.

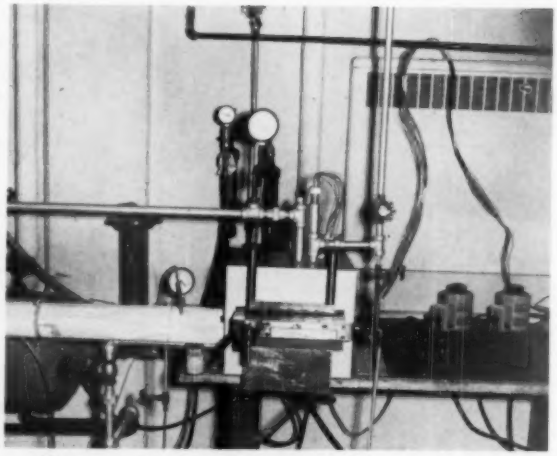

Figure 1. Front view of dry heat thermoresistometer.

In hot-gas heating the gas stream must be distributed so that each cup is exposed to gas where the over-all heating effect of temperature and velocity is the same.

Cup temperatures were measured using 30-gage thermocouples soldered to the inside of the cup bottom. Thermocouple wires were looped away from the cup (Figure 3) and then brought out through the center of the pistons. This insured an isothermal wire length of about 1.0 inch, eliminating, from a practical standpoint, temperature errors resulting from the conduction of heat along the wires. It was necessary to remove the cup supporting mechanism in order that the thermocouple wire could be threaded through these pistons; ears were soldered onto the 5 sample cups so that they could be hung on the cup guide wire (Figure 3). Through trial and error design of the gas manifold, it was possible to have all 5 cups within a temperature range of less than 1.0° F; however, it was impossible to find a reliable correlation between cup temperature and inlet or wall temperatures. Figure 4 shows the interior of the exposure chamber and the distributing manifold. The direction of flow of the gas from the inlet manifold has a definite effect on the temperature pattern of the 5 cups.

Wall temperatures are controlled by 3 manually-operated variable transformers that supply energy to the electric heaters. Wall temperatures are measured by thermocouples attached to

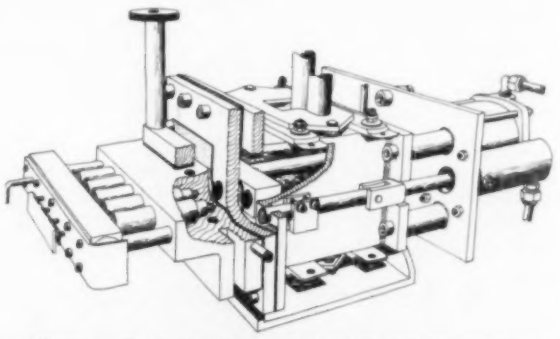

Figure 2. Cut-away drawing of the dry heat thermoresistometer showing the relation of the pistons and inside seal bar.

Figure 3. Sample carrying pistons and operating bar (this unit can be removed from machine in less than 5 min). Special sample cups fitted with thermocouples are in position at the end of the pistons.

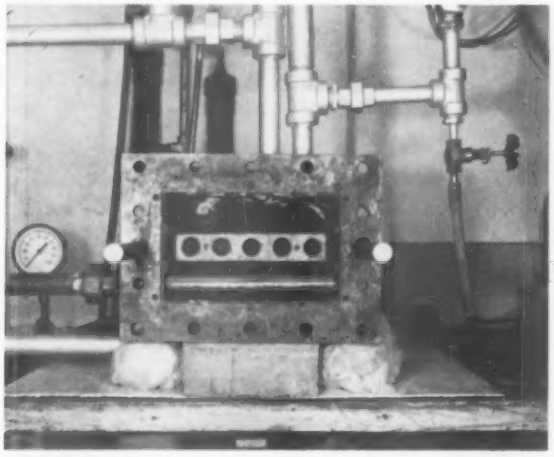

Figure 4. Interior of thermoresistometer exposure chamber showing gas distributing manifold, wall thermocouples and seal bar.

the inside walls of the apparatus. Wall temperature input requirement varies with exposure temperature, room temperature, sample heating time, and temperature difference between wall temperatures and gas control temperature.

The problem of determining the temperature of the cups during actual testing was solved by reducing the number of cups per test from 5 to 4 and leaving one cup, fitted with a thermocouple similar to those described above, in the machine at all times. This thermocouple, connected to a temperature recording potentiometer, gives a complete temperature record of this one cup. Since the range of variation between cups has previously been established to be less than 1.0° F, all cups will always be within ± 1.0° F of the temperature of the cup with the thermocouple.

Lag correction factor. Heating characteristics of sample cups were determined in both air and superheated steam for gas flow rates ranging from TRP's (thermoresistometer pressure) of 5 to 20 in of water; the normal operating TRP is 10 to 15 in of water. TRP, used as a measure of flow for convenience, is the inches of water pressure necessary to force the gas flow volume through the discharge orifice. The relationship of TRP with flow rate of air under standard conditions is illustrated in Figure 5. Cups in all 5 positions were tested and each test was replicated 3 times for each variable. Data were plotted and the heating rate (f_h) and time to reach 1.0° F below heating medium (B) determined. [Terms are defined and the method of plotting data given in Ball and Olsen *(2)*]. Initial temperature of the cups was 90 ±3° F below heating medium temperature.

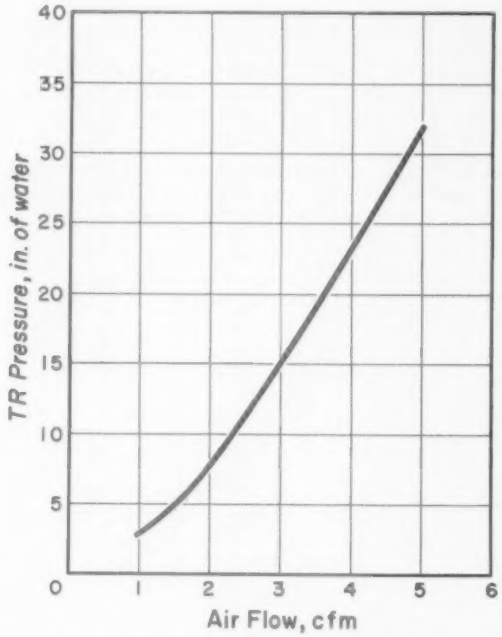

Figure 5. Relationship of exposure chamber pressure and gas flow rate.

Values of f_h and B were plotted as a function of TRP and a straight line was drawn by eye through each set of data. Values of f_h and B for a TRP of 5 and 20 obtained from these curves are listed in Table 1.

The lethality accumulated during heating, U, to 1.0° F below the heating medium temperature was calculated, using the table of functions of Hicks *(4)* for z values of 30, 40, 50, and 60° F assuming a straight line semi-logarithmic heating rate and a logarithmic rate of bacterial destruction. The heating correcion is B minus U.

The cooling correction is based on the assumption that no appreciable cooling occurs until the cups touch the recovery liquid, whereupon the surface temperature is instantly reduced to a point at which the lethal effect is negligible. The correction was calculated to be 0.015 min, the time required for the samples to be withdrawn from the heating chamber and dropped into the recovery medium and be quenched. The cooling correction is the same for all values of z.

The total lag correction, algebraic sum of the heating and cooling corrections, is shown graphically in Figure 6 as a function of z and TRP.

Dry heat thermal resistance studies. Bacterial spores of a Curran's strain 15 μ of the facultative mesophile *Bacillus subtilis*[c] was used as the test organism. The identification number 5230 used by Schmidt was retained. Cultures were grown on slants of nutrient agar containing 1 ppm manganese; the slants were incubated at 99° F (37° C) for 5 to 7 days before harvesting the spores. The washed and filtered spores were suspended in M/15 phosphate buffer (pH of 7.0) and

TABLE 1

Average heating rates of sample cups in air and superheated steam

	TRP	f_h	B
	in of water	*min*	*min*
Air	5	0.39	0.68
	20	0.26	0.48
Superheated steam	5	0.36	0.62
	20	0.24	0.42

put in sterile 8-oz bottles with glass beads. The spores were counted by plating on Dextrose-Tryptone-Starch Agar after a 15 minute heat shock at 212° F (100° C). The inoculating suspension was prepared by diluting the original spore suspension with M/15 phosphate buffer to give approximately 10^8 spores per ml (actual count 1.1 x 10^8 spores per ml). Cleaned tin plate TDT cups were placed in a glass petri dish, the cups and dish sterilized in hot air, after which 0.01 ml of the inoculating suspension was placed in each cup, using a micropipette. Samples were dried to remove the free water. Initially a vacuum oven operated at a temperature of 95° F (35° C) was used; however, the petri dishes containing the loaded cups were placed in a desiccator with a drying agent in later studies. The samples remained in the desiccator, which was stored in a refrigerator at 40.0° F (4.4° C) until needed.

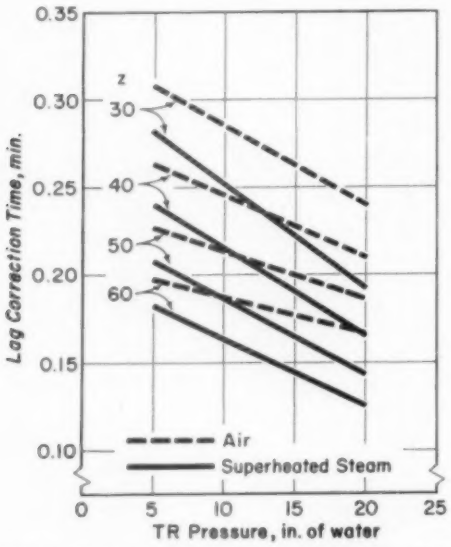

Figure 6. Lag correction time as a function of the exposure chamber pressure.

Dextrose-Tryptone-Starch broth with 0.04% BCP indicator was used as the subculture medium. Positive tubes were those containing acid as evidenced by a color change and the characteristic pellicle. The subculture tubes were incubated at 99° F (37° C) for 4 weeks.

Only 4 cups per test were used in the several preliminary dry-heat studies (including the one reported below); however, 8 cups (2 runs of 4 cups each) are being used in studies now in progress.

Data were evaluated using the probability method of Schmidt *(8)*; both LD50 and D values were calculated. (LD50 is defined as the time in minutes when 50% of the samples tested would be negative; D value is defined as the time in minutes for a 90% reduction in the number of organisms at a given temperature.) The 95% confidence interval was calculated where sufficient data were available. Use of LD50 values [suggested by Schmidt *(8)*] is appropriate for these data since it is desirable to obtain an accurate comparison of the values at the different temperatures without having to resort to D values.

Wet-heat thermal resistance studies. Wet-heat (saturated steam) thermal resistance studies were made over the temperature range of 241 to 272° F (116 to 133° C) with the same spore suspension and recovery medium as used in the dry heat studies. Studies made by Anderson *(1)* indicated that reliable wet-heat results can be obtained with dried spores. A total of 10 cups was exposed at each time-temperature interval (2 runs of 5 cups each) using the apparatus described above.

Data were analyzed using the probability method described by Schmidt *(8)* and the results were expressed as D values.

[c] Obtained from C. F. Schmidt, Continental Can Co., Inc., Chicago.

RESULTS AND DISCUSSION

Results of thermal resistance tests of 5230 in superheated steam as LD50 points, their 95% confidence limits, and the corresponding D values are presented in Figure 7. A straight line was drawn to represent these data since it appears that the logarithm of time varies linearly with temperature. The LD50 line has a z of 42° F and a $LD50_{350}$ of 2.4 min; the D_{350} is 0.57 min. The 95% confidence limits of the LD50 points permit z values from 36° to 48° F. Dotted lines are used in Figure 7 to indicate (a) that there are not sufficient data available at this time to substantiate that the relationship is linear and (b) that justification in the form of death rate data to calculate meaningful D values is not possible at present. The D curve is illustrated to show the range of values that can be expected and for comparison with the wet-heat data in Figure 8.

Results of thermal resistance tests of 5230 in wet heat are presented in Figure 8. The D curve has a z of 14.8° F and a D_{250} of 0.35 min.

Variation in LD50 values and the wide confidence interval appear to be normal for dry heat in contrast to the behavior of wet-heat data illustrated in Figure

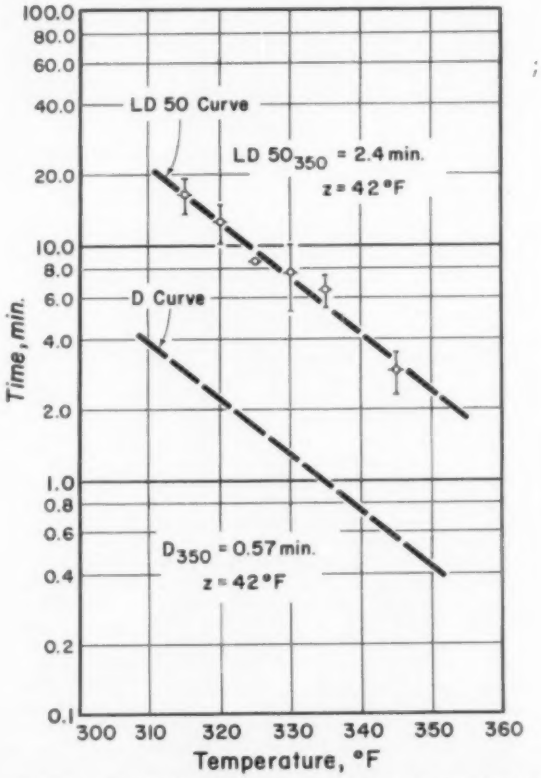

Figure 7. Thermal resistance curve for 5230 in superheated steam.

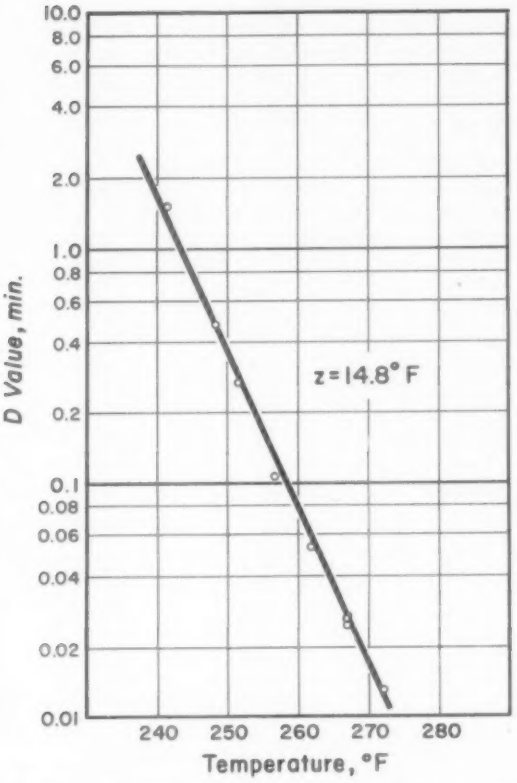

Figure 8. Thermal resistance curve for 5230 in saturated steam.

8. It is acknowledged that use of 8 or 12 cups per test is more desirable and could reduce the confidence interval.

The operation of the equipment, in use since December 1957, has been satisfactory. Dry heat is more difficult to use than wet-heat due to the more complicated temperature control and the necessity of allowing the equipment to reach steady state conditions before testing can begin.

SUMMARY

An apparatus is described that makes possible the study of the dry-heat resistance of microorganisms under controlled conditions of time and temperature in the range 300 to 380° F (149 to 193° C). The accuracy of the temperature control system is ± 1.0° F and time ± 0.1 minutes. The lag correction factor of the sample cups has been evaluated as a function of gas flow for both air and superheated steam. The dry-heat resistance of spores of organism 5230 (similar to *B. subtilis*) to superheated steam has been determined and it was found to have an $LD50_{350}$ of 2.4 min and a z of 42° F.

Acknowledgment

The author wishes to acknowledge and to extend his thanks to Dr. C. F. Schmidt and J. H. Bock of the Metal Division, Research and Development, Continental Can Co. Inc. for their inspiration, assistance, and tolerance over the several years that we have worked together in this effort; to Mr. L. T. Deason of the Adams Tool Company, Lansing, who made valuable suggestions in the design of the machine; and also to Dr. R. N. Costilow, Mr. T. E. Anderson, Mr. R. H. Wilkinson and Mr. Allen Stewart of Michigan State University, who have helped on this project.

The financial assistance of the Continental Can Co., Inc. in making this project a reality is gratefully acknowledged.

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Food Additives Symposium

The past year was an eventful one in the field of food additives. Aminotriazole, stilbestrol, coal tar colors, and the Delaney clause are names which came into common use and aroused public interest and concern in food additive problems.

In many ways, however, the past year was not a decisive one. The Food Additives Amendment became fully effective in March, 1960, but decisions concerning the status of large numbers of food additives were postponed through one-year extensions. Basic problems raised by the Delaney clause, the practical equivalent of zero, and general recognition of safety remain to be answered.

Perhaps the past year might best be characterized as a period of appraisal, a time during which industry and government both have sought to determine the impact of the new food additive regulations in order that their present operations and future plans be adjusted in the most effective fashion.

As in the past several years, the Food Additives Committee of IFT has invited outstanding speakers to discuss at the Annual Meeting the current status of several aspects to the food additives problem. The following papers were presented at the Food Symposium held in San Francisco in May, 1960.

J. F. Mahoney, Chairman
Food Additives Committee

Recent Progress in the Consideration of Flavoring Ingredients Under the Food Additives Amendment*

Richard L. Hall
McCormick & Company, Inc., Baltimore 2, Maryland

THIS MEETING demonstrates again that we believe it is helpful to exchange problems and what we hopefully regard as "solutions." In this paper I shall outline a few of the principal current problems facing the flavor industry under the Food Additives Amendment, and tell something of our efforts to cope with them. While some of our problems are unusual and peculiar to our industry, others are of direct general concern. The activities described below are those of the Food Additives Committee of the Flavoring Extract Manufacturers' Association, and of the companies that support and participate in our Committee's program.

One point should be made clear at the beginning. The Food Additives Amendment places on the Food and Drug Administration an enormous burden. It not only confers a large increase in responsibility, but it does so in areas where the terrain and boundaries are not fully known and are therefore not clearly defined. The Amendment is, in that respect, somewhat like a colonial land grant—broad, inexact, and conferring large powers in an area only slightly explored. Congress wisely contented itself with writing, for the most part, only general rules of conduct. These, to be effective, must be applied sensibly to the actual circumstances as they develop and are more fully understood. For the most part, we in the flavor industry believe that the FDA has discharged well its initial responsibilities. We have some substantial differences of opinion with the FDA, primarily concerning matters of interpretation and administrative policy. We are compelled to report our concern at what seems to us an unfortunate tendency to subordinate its scientific judgment to administrative convenience and political appeal. In this paper, I shall mention in detail some of these points on which we differ. But these differences should not be allowed to obscure our whole-hearted approval of most of the Food Additives Amendment and of the FDA's principal measures to enforce it. We may reasonably say that, if it were our decision, we would in certain respects, act differently and more wisely. But we should also hope that we would, in other respects, perform as well.

Last year we distributed a questionnaire which formed the basis of a Flavor Additive survey sponsored by the FEMA. Many of you will recall, doubtless with some distaste, that it covered approximately 1100 substances. We gave the name of each, and a structural formula, if known. We asked the flavors in which it is used, degree of importance, earliest date of use, level in ppm introduced into the finished food, approximate annual volume used, and toxicity data where available. The original questionnaire went to all flavor manufacturers and to a cross-section of the food processing industry. We received, on the whole, an excellent response. One hundred seventy completed forms were returned. We have now imposed on your patience by sending out an addenda section to those firms which, by completing the original ques-

* Presented at the Twentieth Annual Meeting of the Institute of Food Technologists, San Francisco, California, May 16, 1960.

tionnaire, showed that they were useful sources of information.

Early this year, it became apparent that we could not possibly complete the tabulation and evaluation of results of our survey before March 5th, when the Amendment was scheduled to become fully effective. Because the Amendment does not apply to those substances which are "generally recognized as safe" (GRAS), technically no extension or extension application was necesary for them. But the classification of what is GRAS, and even the definition of GRAS, were not and are not settled, so it seemed wise to provide unequivocally for the continued use of almost all flavor ingredients by including each of them, specifically, in an extension request. This was done with the clear understanding that this would in no way prejudice a later determination that the substances were GRAS.

CLASSIFICATION OF THE SUBSTANCES

During the past months, our committee has tabulated the questionnaire and reviewed the results. We have classified each substance into one of 4 categories. These are:

(1) No further action (NFA)
(2) Food
(3) GRAS
(4) Food Additive

A number have been held temporarily in suspense pending receipt of further information. The complete listing to date of our committee's action on each substance will be found at the end of this article.

We have classified as NFA, those items which received so scanty a response that it appeared they had little or no use in food. We labeled as Food, a number of substances which seemed to be major rather than minor ingredients, or nutrients rather than modifiers. We regarded as GRAS those substances which satisfied one or more of the following numbered requirements:

(1) Natural occurrence in a food,
(2) Known ease of metabolization in the body (in the quantity and at the dietary level employed),
(3) A close structural relationship to another substance known to be safe,
(4) A long history of widespread and apparently safe use, (e.g., thirty years by several companies), or
(5) Simultaneous fulfillment of all of the following qualifications:
   a. In use for at least 10 years by more than one company,
   b. Average maximum use level in any finished food not in excess of 10 ppm,
   c. Annual national consumption of less than 1000 lb,
   d. Absence of any unfavorable indications from structure, composition, or experience in use, which would cast doubt on its safety.

An ingredient which conforms to these four restrictions is, we feel, toxicologically insignificant under the conditions of intended use, (and hence GRAS).

If a substance meets any of the 5 foregoing requirements, this is, in our view, an adequate basis for judging it to be GRAS. If it is not GRAS, then, by law, it is a food additive and we have so classified those ingredients which are in use, and which do not fit into any GRAS category.

The fact that we have classified a substance, perhaps temporarily, as a food additive, does not mean that the substance is unsafe. It merely means that we do not have sufficient information, at this time, to permit us to feel that it is GRAS.

We have transmitted this information, as it has been developed, to the FDA. They propose to classify these ingredients into 7 groups and deal with them as follows:

1. GRAS by prior publication in the *Federal Register*
   No action required
2. Used under a prior sanction
   Publish in *Federal Register*
3. Safe and believed to be GRAS
   Propose as GRAS in *Federal Register*
4. Safe, but lacking general recognition of safety
   a. Extension
   b. Industry petition — no pharmacology — sufficient information to establish safe usage.
   c. Regulation
5. Probably safe; no undue risk to the public health
   a. Extension
   b. Industry petition with sufficient pharmacology and chemistry to establish safe usage
   c. Regulation
6. Undue risk to public health
   a. No extension
   b. Petition may be considered
   c. Regulation may be considered
7. No interest in industry
   Disregard for the present

Our only major disagreement, at present, with this tentative classification concerns the Administration's basis for regarding a substance as GRAS.

All of the substances in FDA's Group No 4 are unquestionably safe, but the FDA is unwilling to propose publicly that they are generally recognized as safe. Therefore, they prefer to handle them by the petition route, but omitting the requirements for animal test data and an analytical method. We believe that to call a safe ingredient a food additive, under the law and in the present state of public opinion, subjects it to doubt and suspicion, and to the danger of banishment. It is food technology's equivalent of taking the Fifth Amendment. We also feel that to file a petition which ignores completely several clear-cut statutory requirements would commit us to a danger-

ous course of action, resulting at best in a probably invalid regulation.

RECOGNITION OF SAFETY

This brings us to a consideration of what constitutes general recognition of safety. The Amendment defines a substance as a food additive "if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures . . . or experience based on common use in food . . . to be safe under the conditions of its intended use . . . " Many persons, including FDA officials, have pointed out that the law confers no special authority on the FDA with respect to deciding that an ingredient is GRAS. Anyone, on adequate evidence, may so decide.

To date the FDA has taken the position that this general recognition must take place among a large group from the appropriate scientific disciplines. If the food use of a substance is not known to a substantial segment of the scientific community, then it is not "generally recognized as safe."

We, on the other hand, believe that the law's singular use of the word "its" in the phrase "qualified . . . to evaluate its safety" is important, not trivial, and intentional, not fortuitous. This general recognition is to take place not among some large group in the scientific community who may have slight if any contact with, or knowledge of, the use of a particular ingredient in food. Rather, the recognition of safety must be among those who have the necessary training and experience—pertinent experience. This clearly implies direct and accurate knowledge of the nature of the substance itself and of the conditions of its intended use.

Perhaps an analogy may make this point more clear. Dr. "X" has his Ph.D. in archeology from Columbia. He has taught archeology and paleontology for 10 years at Northwestern. After 7 field trips to the Southwest, 6 articles, 2 monographs and a textbook (used only in his graduate course) he is an acknowledged authority on Indian archeology of the American Southwest. Does this qualify him to judge the authenticity and date of a Sumerian clay tablet, or a fossilized jaw bone from South Africa, or an Etruscan spear point? Clearly not. Professional education and exercise of appropriate skills are not enough. They are necessary but not sufficient conditions. They must be coupled with detailed knowledge of the specific area and item in question, and this combination may be found only in a few persons.

We are quite certain that the experts qualified in the case of many flavoring substances are limited at present to those in the FDA and our own committee who have access to the data we have gathered. For this reason, we are making public our tentative classification.

Now we recognize that a broader base of judgment than our committee alone, may be desirable. We have therefore begun to establish a separate, independent panel of 6 to 8 acknowledged experts in toxicology and pharmacology to whom we shall refer those items enjoying significant food use and which are not already recognized as GRAS by the FDA. We shall supply to this panel all the information we have. This will be no mail-order panel of anonymous and dubiously qualified "experts." Our panel will meet as a group to exchange views, obtain further information, and advise our committee on its conclusions. If the panel unanimously feels that a substance is safe, if this concurs with the view of our committee, and if the FDA has no reservations regarding safety, then, in our view, this fulfills completely the statutory concept of GRAS.

We shall make our data available to the other qualified and interested groups such as the Food Protection Committee of the NRC, and we welcome their considered judgment in this area.

Even though, by this means, we establish GRAS status for a large number of important ingredients, there will still be many others which will require some data from animal testing in order that we may be assured of their safety under conditions of intended use. By and large, these data are lacking for the great body of flavoring ingredients. The problem of obtaining this information on so large a number of additives is complicated by the fact that several, and sometimes many, manufacturers may use a given substance and yet the annual volume will often be far less than 1000 lb per year in total food use within the United States. Thus, many of these additives do not in themselves have a commercial value sufficient to justify chronic or long term studies. Even short term or sub-acute studies can feasibly be undertaken only as a joint effort by all interested parties.

FINANCING THE TESTS

We believe that the only practical method of raising the necessary funds is to obtain them from those firms that are the principal manufacturers and users of these additives. Although many other firms use these products indirectly, they often do so without knowing that they use them. There seems no reasonable method of making such companies directly share the burden in proportion to their unknown share of the benefit.

We have, therefore, devised the following procedure as a means of arranging the financing of tests on additives whose importance justifies the necessary expenditure.

1. Our FEMA Food Additives Committee will prepare a list of those additives on which at least short term toxicity data are needed. This list will show for each substance:
   a. the total number of firms reporting use of it
   b. the over-all importance rating received
   c. a rough estimate of the annual volume used in flavors
   d. an estimate of the approximate cost of the toxicity tests proposed for each item.
2. The list described above will be sent by the Executive Secretary of the FEMA to each company which participated in our survey. Each

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firm will be requested to indicate, by individual item, the amount of money it is willing to subscribe for the proposed tests on each substance.

3. The lists will be collected and totaled. Where a particular substance is over-subscribed, the amount "pledged" by each firm will be reduced in proportion, and each subscriber thereto informed of its remaining overage. All companies will be told those additives for which an adequate total has not been pledged and the amount which remains to be collected. They will thus be asked to reallocate their overages or otherwise increase their commitments, if, in their judgment, the importance of the substance to them justifies the expenditure. After a series of such readjustments, our Executive Secretary's office will develop a final listing of substances for which the proper amount has been subscribed. Each firm will know how much it will owe on each item and will be billed accordingly as the tests are run. Those additives which do not receive sufficient support will inevitably be dropped. The financial records will be confidential, and withheld even from our committee.
4. The evidence from the animal feeding tests will be reviewed by our FEMA committee, its expert panel and the FDA. We expect that in a high percentage of cases the information will permit the filing of a petition and the issuance of a regulation providing for the safe use of the substance in question. We also hope that this further evidence of safety, if given sufficient distribution, will eventually result in general recognition of safety, rather than in perpetual treatment of the ingredient in question as a food additive.

From the foregoing it is obvious that we cannot complete our program on all ingredients prior to March 5, 1961. Certainly those substances which must undergo a toxicity testing program will be delayed. We shall request, we hope with the collaboration of the Administration, a statutory extension of the final deadline for those substances on which experimental work and evaluation is not yet complete.

THE DELANEY CLAUSE

The flavoring industry is seriously concerned by the Administration's attitude toward the so-called "Delaney Clause" of the Food Additives Amendment. Comments of prominent FDA officials, soon after the last-minute inclusion of the clause in the bill, seemed to show they felt the clause was unnecessary, but also unimportant. Unfortunately, later developments have not confirmed this happy view. Our committee agrees with the many previous comments which have pointed out so forcefully that the clause is unnecessary, arbitrary, and restores to the law the same pernicious *per se* concept which it was a primary objective of the Food Additives Amendment to eliminate. We feel in addition, that it is self-defeating. It shuts off by arbitrary congressional action, the principal source of further information on the subject of dietary carcinogenisis. The defense usually advanced for the Delaney clause is that we "do not know how to set tolerances for carcinogens." But by far the most promising method for determining how to set a tolerance for a carcinogen lies in extensive animal testing and related investigations. These expensive procedures, easily costing several hundred thousand dollars for a single substance, can be undertaken by industry only if some consequences of commercial value may reasonably be expected to ensue.

If utilization of the results of such tests is prohibited, then there is no reason to commence or complete them. It is futile to suggest that, if the tests successfully show that safe levels can be set, then industry can petition Congress to change the Amendment. The difficulties and costs of animal testing are now so great that they are often the fatal bar to further product development. If the requirement of changing a Federal law is added to them, tests will never be started, or will be abandoned as soon as the first hint of carcinogenic action appears.

Thus, the Delaney clause not only locks the door, but throws the key away. It forecloses a solution of the very problem it over-emphasizes. We do not object to closing the door, but we do believe that the Secretary should keep the key, whether he wishes to or not. We think that each of us as individuals, and the firms of which we are a part, should apply whatever influence we can exert toward a more realistic revision of the Delaney clause. Both public education and direct representation to our congressmen are needed.

The flavor industry serves with its products almost every other area within the food field. Because of the variety of our ingredients, their complexity, total number, and low volume, we have in intensified form, most of the problems which the rest of the industry also faces. We hope this partial review of them and of our methods of dealing with them will be of some value to others. We also invite your suggestions and criticism.

FEMA COMMITTEE ACTION ON FLAVOR INGREDIENTS

In examining the list of flavor ingredients that follows, the reader will need to bear in mind that

NFA = No Further Action
GRAS = Generally Recognized as Safe
RMUL = Recommended Maximum Use Level
FA = Food Additive

Absinthium	NFA	Adipic Acid	GRAS
Acacia, Gum	GRAS	Agar-Agar	GRAS
Acetal	GRAS	Alfalfa, Extract	GRAS
Acetaldehyde	GRAS	Algin	GRAS
Aceptanisol	GRAS	Alginates, Sodium, Calcium	
Acetic Acid	GRAS	and Ammonium	GRAS
Acetol Acetate	GRAS	Alkanet Root, Extract	GRAS
Acetophenone	GRAS	Allspice	GRAS
Acetyl *iso*-Butyryl	GRAS	Allspice, Oil	GRAS
Acetyl Cedrene	NFA	Allspice, Oleoresin	GRAS
Acetyl *iso*-Eugenol	GRAS	Allyl Anthranilate	GRAS
Acetyl Methyl Carbinol	GRAS	RMUL 3 ppm	
Acetyl Nonyryl	GRAS	Allyl Butyrate	GRAS
Acetyl Propionyl	GRAS	RMUL 2 ppm	
Acetyl Tributyl Citrate	NFA	Allyl Caprylate	GRAS
Acetyl Valeryl	GRAS	RMUL 5 ppm	
Acetylvanillin	GRAS	Allyl Cinnamate	GRAS
Aconitic Acid	GRAS	RMUL 3 ppm	

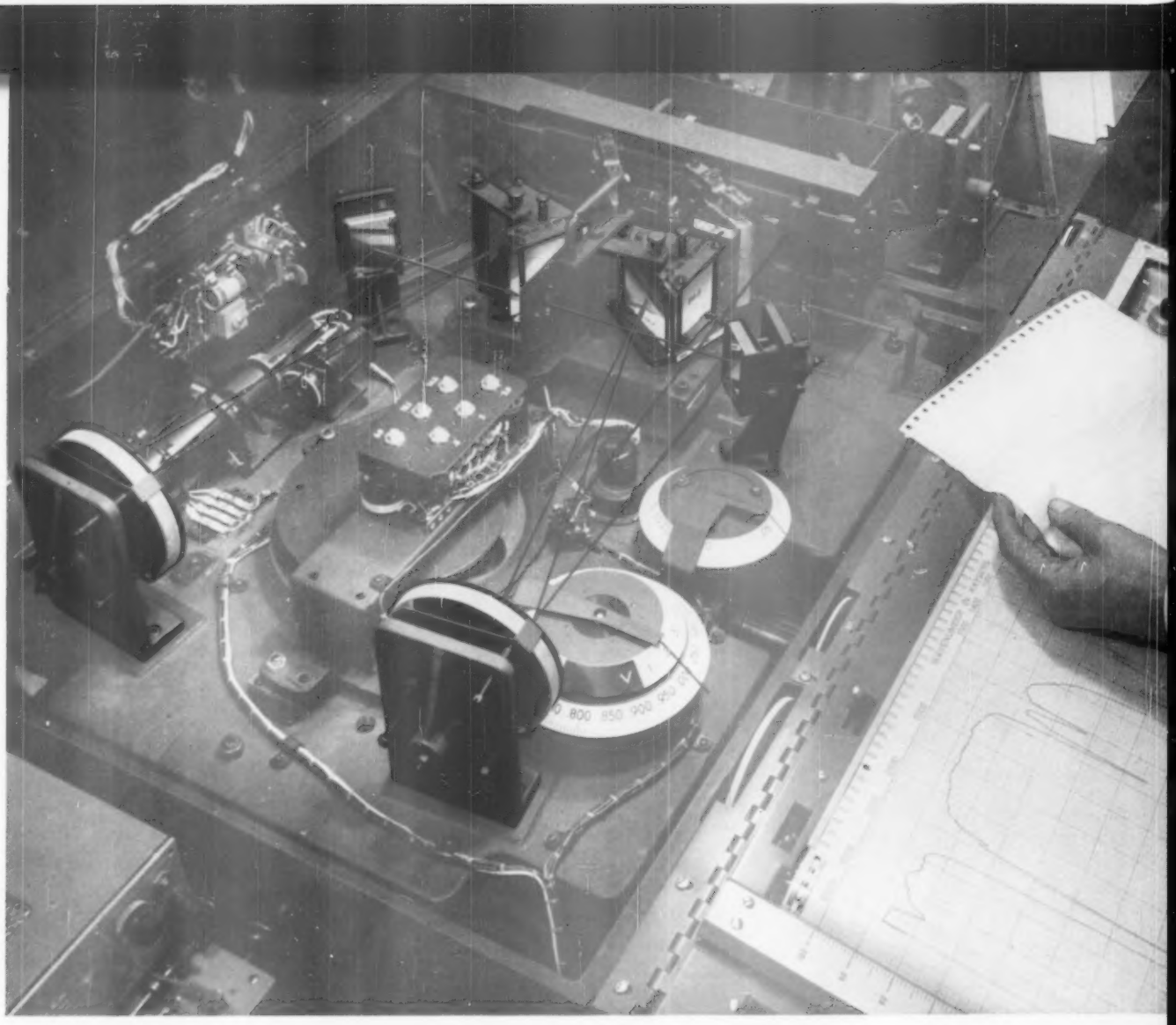
Allyl Cyclohexaneacetate RMUL 4 ppm GRAS
Allyl Cyclohexanebutyrate RMUL 10 ppm GRAS
Allyl Cyclohexanecaproate RMUL 8 ppm GRAS
Allyl Cyclohexanepropionate RMUL 10 ppm GRAS
Allyl Cyclohexanevalerate RMUL 5 ppm GRAS
Allyl 2-Ethyl Butyrate RMUL 2 ppm GRAS
Allyl Furoate RMUL 2 ppm GRAS
Allyl Heptylate RMUL 10 ppm GRAS
Allyl Hexadienoate RMUL 3 ppm GRAS
Allyl Hexanoate GRAS
Allyl Ionone GRAS
Allyl Mercaptan RMUL 2 ppm GRAS
Allyl Nonate RMUL 5 ppm GRAS
Allyl Phenoxyacetate RMUL 2 ppm GRAS
Allyl Phenylacetate RMUL 15 ppm GRAS
Allyl Propionate RMUL 10 ppm GRAS
Allyl *iso*-Thiocyanate GRAS
Allyl Tiglate GRAS
Allyl Undecylate RMUL 1 ppm GRAS
Allyl *iso*-Valerate RMUL 10 ppm GRAS
Almonds, Bitter, Oil (FFPA) GRAS
Almond, Sweet, Oil Food
Aloe Extract GRAS
Althea Root GRAS
Amber Grains, Extract NFA
Ambergris, Tincture GRAS
Ambrette, Absolute, Oil GRAS
Ambrette, Seed, Oil GRAS
Ambrette, Tincture GRAS
Ammonium *iso*-Valerate GRAS
iso-Amyl Acetate GRAS
n-Amyl Alcohol GRAS
iso-Amyl Alcohol GRAS
iso-Amyl Benzoate GRAS
iso-Amyl Benzyl Ether GRAS
n-Amyl Butyrate GRAS
iso-Amyl Butyrate GRAS
n-Amyl Caproate GRAS
iso-Amyl Caproate GRAS
n-Amyl Caprylate GRAS
iso-Amyl Caprylate GRAS
iso-Amyl Cinnamate GRAS
α-*n*-Amyl Cinnamic Aldehyde GRAS
α-Amyl Cinnamic Aldehyde Dimethyl Acetal GRAS
α-(*n*-Amyl) Cinnamyl Acetate GRAS
α-Amyl Cinnamyl Alcohol GRAS
α-Amyl Cinnamyl Formate GRAS
α-Amyl Cinnamyl *iso*-Valerate GRAS
Amyl *p*-Cresyl Ether GRAS
Amylcyclopentenolone GRAS
iso-Amyl Ether GRAS
n-Amyl Formate GRAS
iso-Amyl Formate GRAS
α-*iso*-Amyl Furfurylacetate GRAS
α-*iso*-Amyl Furfuryl acrylate GRAS
α-*iso*-Amyl Furfuryl propionate GRAS
Amyl Furoate GRAS
Amyl Heptoate GRAS
iso-Amyl Laurate GRAS
iso-Amyl Pelargonate GRAS
iso-Amyl Phenylacetate GRAS
iso-Amyl Propionate GRAS
iso-Amyl Pyruvate GRAS
iso-Amyl Salicylate GRAS
iso-Amyl *iso*-Valerate GRAS
Amyris, Oil GRAS
Anethole GRAS
Angelica, Root, Extract GRAS
Angelica, Root, Oil GRAS
Angelica, Seed, Extract GRAS
Angelica, Seed, Oil GRAS
Angelica, Stem, Oil GRAS
Angostura, Extract GRAS
Anisaldehyde GRAS
Anise GRAS
Anise, Oil GRAS
Anise, Star GRAS
Anise, Star, Oil GRAS
Anisole GRAS
Anisyl Acetate GRAS
Anisyl Alcohol GRAS
Anisyl *n*-Butyrate GRAS
Anisyl Formate GRAS
Anisyl Propionate GRAS
Annatto, Extract GRAS
Annatto, Seed GRAS
Apricot Kernel Oil GRAS
Apricot, Oil GRAS
Asafoetida Fluid Extract GRAS
Asafoetida, Gum GRAS
Asafoetida, Oil GRAS
Ascorbic Acid GRAS
iso-Ascorbic Acid GRAS
Ash Bark, Prickly, Extract GRAS

Balm Leaves, Extract NFA
Balsam, Copaiba GRAS
Balsam, Fir, Oil GRAS
Balsam, Fir, Oleoresin GRAS
Balsam, Peru GRAS
Balsam, Peru, Oil GRAS
Basil GRAS
Basil, Oil GRAS
Basil, Oleoresin GRAS
Bay GRAS
Bay, Laurel, California Oil FA
Bay, Leaves, Extract GRAS
Bay, Leaves, Oil GRAS
Bay, Leaves, Oleoresin GRAS
Bay, Sweet, Oil GRAS
Beechwood, Creosote GRAS
Benzaldehyde GRAS
Benzaldehyde Dimethyl Acetal GRAS
Benzaldehyde Propylene Glycol Acetal GRAS
Benzal Glyceryl Acetal GRAS
Benzodihydropyrone GRAS
Benzoic Acid GRAS
Benzoin GRAS
Benzoin, Gum GRAS
Benzophenone GRAS
Benzyl Acetate GRAS
Benzyl Acetoacetate GRAS
Benzylacetone FA
Benzyl Alcohol GRAS
Benzyl Benzoate GRAS
Benzyl *iso*-Butyl Ketone GRAS
Benzyl Butyrate GRAS
Benzyl *iso*-Butyrate GRAS
Benzyl Cinnamate GRAS
Benzyldimethyl Carbinyl Acetate FA
Benzyldimethyl Carbinyl *n*-Butyrate GRAS
Benzyldimethyl Carbinyl Formate GRAS
Benzyl Dipropyl Ketone GRAS
Benzylethyl Alcohol GRAS
Benzyl Ethyl Ether GRAS
Benzyl-*iso*-Butylcarbinol NFA
Benzyl *iso*-Eugenol GRAS
Benzyl Formate GRAS
Benzyl Mercaptan GRAS
Benzyl Methoxethyl Acetal GRAS
Benzyl Methyl Tiglate FA
Benzyl Phenylacetate GRAS
Benzyl Propionate GRAS
Benzyl Propyl Carbinol NFA
Benzyl Salicylate GRAS
Benzyl *iso*-Valerate GRAS
Bergamot, Oil GRAS
Bilberry Extract NFA
Birch, Sweet, Oil GRAS
Birch, Tar, Oil, Refined (Rectified) GRAS
Blackberry, Bark, Extract GRAS
Blessed Thistle Herb NFA
Blessed Thistle Herb, Extract NFA
Blessed Thistle Herb, Extract, Solid NFA
Blessed Thistle Herb, Oil NFA
Bois De Rose, Oil GRAS
Borneol GRAS
iso-Borneol GRAS
Bornyl Acetate GRAS
iso-Bornyl Acetate GRAS
Bornyl Formate GRAS
iso-Bornyl Formate GRAS
iso-Bornyl Propionate GRAS
Bornyl Valerate GRAS
Bornyl *iso*-Valerate GRAS
iso-Bornyl *iso*-Valerate GRAS
Boronia, Absolute GRAS
Brominated Vegetable Oils GRAS
Bruit, Oil NFA
Buchu, Leaves, Oil GRAS
Buck Bean Leaves, Extract NFA
n-Butanol GRAS
iso-Butanol GRAS
Butter Acids GRAS
Butter, Esters GRAS
Butter Starter Distillate GRAS
n-Butyl Acetate GRAS
iso-Butyl Acetate GRAS
n-Butyl Acetoacetate GRAS
iso-Butyl Acetoacetate GRAS
iso-Butyl Angelate NFA
n-Butyl Anthranilate GRAS
iso-Butyl Anthranilate GRAS
iso-Butyl Benzoate GRAS
iso-Butyl Benzyl Carbinol FA
Butyl Benzyl Ether FA
n-Butyl Butyrate GRAS
iso-Butyl Butyrate GRAS
n-Butyl *iso*-Butyrate GRAS
iso-Butyl *iso*-Butyrate GRAS
Butyl Butyryl Lactate GRAS
n-Butyl Caproate GRAS
iso-Butyl Caproate GRAS
n-Butyl Cinnamate GRAS
iso-Butyl Cinnamate GRAS
α-(*n*-Butyl) Cinnamic Aldehyde GRAS
n-Butyl Decylenate GRAS
n-Butyl Formate GRAS
iso-Butyl Formate GRAS
iso-Butyl Furyl Propionate FA
Butyl Heptylate GRAS
iso-Butyl Heptylate GRAS
Butyl *p*-Hydroxybenzoate GRAS
n-Butyl Lactate GRAS
n-Butyl Laurate GRAS
n-Butyl Levulinate GRAS
iso-Butyl-*n*-Methyl Anthranilate GRAS
p-ter-Butyl Methyl Phenylacetate GRAS
Butyl Oleate NFA
n-Butyl Phenylacetate GRAS
iso-Butyl Phenylacetate GRAS
n-Butyl Propionate GRAS
iso-Butyl Propionate GRAS
6-*iso*-Butyl Quinoline GRAS
ter-Butyl Quinoline NFA
iso-Butyl Salicylate GRAS
Butyl Stearate GRAS
n-Butyl Stearate GRAS
Butyl Undecylenate GRAS
Butyl Valerate GRAS
n-Butyl *iso*-Valerate GRAS
Butylated Hydroxyanisole (BHA) GRAS
RMUL 200 ppm in final food
Butylated Hydroxytoluene (BHT) GRAS
n-Butyraldehyde GRAS
iso-Butyraldehyde GRAS
n-Butyric Acid GRAS
iso-Butyric Acid GRAS
Butyroin GRAS

Cabreuva, Oil GRAS
Cacao, Extract Food
Cactus, Root, Extract GRAS
Cade, Oil (Juniper Tar) GRAS
Caffeine GRAS
Cajuput, Oil GRAS
Calamus GRAS
Calamus, Oil GRAS
Calcium Acetate GRAS
Calumba, Extract NFA
Camphene FA
d-Camphor GRAS
Camphor, Japanese, Oil, White GRAS
RMUL 10 ppm
Camphor, Oil GRAS
Cananga, Oil GRAS
Cape Aloe Extract GRAS
Capers Food
Capsicum Extract GRAS
Capsicum, Oleoresin GRAS
Caramel Color GRAS
Caraway GRAS
Caraway, Oil GRAS
Carboxymethyl Cellulose GRAS
Cardamom GRAS
Cardamom Seed, Oil GRAS
Carmine GRAS
Carob Bean, Extract GRAS
Caryophyllene GRAS
Caryophyllene Alcohol NFA
Carrot, Oil GRAS
Carvacrol GRAS
Carveol GRAS
Carvomenthol NFA
Carvone GRAS
Carvyl Acetate GRAS
Carvyl Propionate GRAS
Cascara Bitterless Extract GRAS
Cascarilla, Bark, Extract GRAS
Cascarilla, Bark, Oil GRAS
Cassia GRAS
Cassia, Bark, Extract GRAS
Cassia, Bark, Oil GRAS
Cassia Buds GRAS
Cassie, Absolute GRAS
Castor, Oil GRAS
Castoreum Extract GRAS
Castoreum, Liquid GRAS
Catechu, Extract GRAS
Catechu, Powder GRAS
Cayenne GRAS
Cedar, Leaf, Oil FA
Cedarwood, American, Red Oil Action not completed
Cedarwood, American, White, Oil FA
Cedrenol NFA
Cedrol NFA
Cedrone NFA
Cedryl Acetate GRAS
Celery Seed GRAS
Celery Seed Extract GRAS
Celery Seed, Extract, Solid GRAS
Celery Seed, Oil GRAS
Centaury Herb FA
Cetyl Alcohol GRAS
Chamomile Flower, English, Oil GRAS
Chamomile Flower, Hungarian, Oil GRAS
Chamomile Flower, Roman, Extract GRAS
Chamomile Flower, Roman, Oil GRAS
Cherry Laurel Water NFA
Cherry Leaf, Extract NFA
Cherry Pits, Extract GRAS
Cherry, Wild, Bark, Extract GRAS
Chervil GRAS
Chestnut Leaves NFA
Chestnut Leaves, Extract NFA
Chestnut Leaves, Extract, Solid NFA
Chevreperylills Essence, Liquid, Absolute NFA
Chicory, Extract GRAS
China Bark, Extract NFA
Chirata Herb, Extract GRAS
Chives Food
Cinchona, Bark, Red GRAS
Cinchona, Bark, Red, Extract GRAS
RMUL 100 pppm
Cinchona, Bark, Red, Oleoresin NFA
Cinchona, Bark, Yellow GRAS
Cinchona, Bark, Yellow, Extract GRAS
RMUL 100 ppm
Cinchona, Bark, Yellow, Oleoresin NFA
Cinchonidine NFA
Cinnamaldehyde GRAS
Cinnamaldehyde Ethylene Glycol Acetal GRAS
Cinnamic Acid GRAS
Cinnamon GRAS
Cinnamon, Bark, Extract GRAS
Cinnamon, Bark, Oil GRAS
Cinnamon, Leaf, Oil GRAS
Cinnamyl Acetate GRAS
β-Cinnamylacryloyl Piperidide GRAS
Cinnamyl Alcohol GRAS
Cinnamyl Anthranilate GRAS
Cinnamyl Butyrate GRAS
Cinnamyl *iso*-Butyrate GRAS
Cinnamyl Cinnamate GRAS
Cinnamyl Formate GRAS
Cinnamyl Phenylacetate GRAS
Cinnamyl Propionate GRAS
Cinnamyl *iso*-Valerate GRAS
Cire D'Abeille Absolute GRAS

Citraconic Acid	GRAS
Citral	GRAS
Citral Diethyl Acetal	GRAS
Citral Dimethyl Acetal	GRAS
Citric Acid	GRAS
Citronella, Oil	GRAS
Citronellal	GRAS
d-Citronellol, *l*-Citronellol	GRAS
Citronellyl Acetate	GRAS
Citronellyl Butyrate	GRAS
Citronellyl *iso*-Butyrate	GRAS
Citronellyl Formate	GRAS
Citronellyl Oxyacetaldehyde	GRAS
Citronellyl Phenylacetate	GRAS
Citronellyl Propionate	GRAS
Citronellyl Valerate	GRAS
Citrus Peels, Extract	GRAS
Civet, Absolute	GRAS
Clary	GRAS
Clary, Sage, Oil	GRAS
Cloves	GRAS
Clove, Bud, Extract	GRAS
Clove, Bud, Oil	GRAS
Clove, Bud, Oleoresin	GRAS
Clove, Leaf, Oil	GRAS
Clove, Stem, Oil	GRAS
Clover Tops, Red, Extract, Solid	NFA
Coca, Leaf, Extract (Decocainized)	GRAS
Cochineal	GRAS
Coffee, Extract	Food
Cognac, Green, Oil	GRAS
Cognac, White, Oil	GRAS
Copaiba, Oil	GRAS
Coriander	GRAS
Coriander, Oil	GRAS
Corn Silk	GRAS
Costus, Root, Oil	FA
Cramp Bark, Extract	GRAS
p-Cresol	GRAS
β-Cresoxyethanol	NFA
β-Cresoxy Ethyl Acetate	NFA
m-Cresyl Acetate	NFA
o-Cresyl Acetate	GRAS
p-Cresyl Acetate	GRAS
p-Cresyl *iso*-Butyrate	GRAS
p-Cresyl Laurate	NFA
m-Cresyl Phenylacetate	NFA
p-Cresyl Phenylacetate	GRAS
m-Cresyl Phenyl Ether	GRAS
p-Cresyl Salicylate	NFA
Cubeba	GRAS
Cubebs, Oil	GRAS
Cudbear Extract	NFA
Cumin	GRAS
Cumin, Oil	GRAS
Cuminal	GRAS
Cuminic Alcohol	GRAS
Cuminyl Acetaldehyde	GRAS
Curacao, Peel, Extract	GRAS
Curacao Peel, Oil	GRAS
Currant Buds, Oleoresin	NFA
Currant Buds, Black, Absolute	GRAS
Currant Buds, Black, Extract, Solid	NFA
Cyclamen Alcohol	NFA
Cyclamen Aldehyde	GRAS
Isocyclocitral	FA
Cyclohexyl Acetate	FA
Cyclohexyl Acetic Acid	FA
Cyclohexyl Anthranilate	FA
Cyclohexyl-*n*-Butyrate	FA
Cyclohexyl Cinnamate	FA
Cyclohexylethyl Acetate	FA
Cyclohexyl Formate	FA
Cyclohexyl Methyl Ether	NFA
Cyclohexyl Propionate	GRAS
Cyclohexyl *iso*-Valerate	FA
Cyclopentanone, Acetylated	FA
Cyclopentenyl Methyl Acetate	FA
p-Cymene	GRAS
Cypress, Oil	NFA
Damar Gum	GRAS
Dandelion Fluid, Extract	GRAS
Dandelion Root, Extract, Solid	GRAS
Davana Oil	GRAS
γ-Decalactone	GRAS
δ-Decalactone	GRAS
Decanal	GRAS
Decanal Dimethyl Acetal	GRAS
Decanoic Acid	GRAS
n-Decanol	GRAS
n-2-Decenal-1	GRAS
n-Decyl Acetate	GRAS
iso-Decylaldehyde	GRAS
Decyl *n*-Butyrate	GRAS
n-Decyl Propionate	GRAS
Deer Tongue, Extract	GRAS
Dextrose	Food
Diallyl Sulfide	GRAS
Diacetyl	GRAS
Diacetyl Monoxime	NFA
Diallyl Disulfide	GRAS
Dibenzyl Ether	GRAS
Dibenzyl Ketone	GRAS
Dibutyl Butyrolactone	FA
Dibutyl Sebacate	GRAS
Dibutyl Sulfide	GRAS
Diethyl Anthranilate	GRAS
Diethyl Malate	GRAS
Diethyl Hydroquinone	NFA
Diethyl Maleate	FA
Diethyl Malonate	GRAS
Diethyl Sebacate	GRAS
Diethyl Succinate	GRAS
Diethyl Tartrate	GRAS
Diglycol Laurate	NFA
Dihydrocarveol	GRAS
Dihydrocarveol Acetate	GRAS
Dihydropyronyl-2-Methyl Acetate	NFA
Dihydrosafrol	FA
Dill	GRAS
Dill, Oil	GRAS
Dill, Seed, Indian	GRAS
1,4-Dimethoxybenzene	FA
Dimethyl Acetophenone	GRAS
Dimethyl Anthranilate	GRAS
Dimethyl Benzyl Carbinol	GRAS
Dimethyl Hydroquinone	FA
Dimethyl Octanol-8(2,6)	GRAS
α,α-Dimethylphenylacetaldehydo	NFA
Dimethyl Phenylethyl Carbinyl Acetate	FA
Dimethyl Resorcinol	GRAS
Dimethyl Succinate	GRAS
Dimethyl Sulfide	GRAS
Dipropyl Ketone	GRAS
Disodium Phosphate	GRAS
Ditetrahydrofurfuryl Phthalate	NFA
Dittany of Crete	GRAS
Dittany of Crete, Extract	NFA
Dittany of Crete, Extract, Solid	NFA
Dittany of Crete, Oil	NFA
γ-Dodecalactone	GRAS
δ-Dodecalactone	GRAS
n-Dodecanal	GRAS
2-Dodecenal-1	GRAS
n-Dodecyl Alcohol	GRAS
Doggrass, Extract	GRAS
Draco Rubin Extract	NFA
Dulse	NFA
Elder Flowers	GRAS
Elecampane, Root, Extract	NFA
Elecampane, Root, Oil	GRAS
Elemi, Gum	GRAS
Elemi, Oil	GRAS
Erigeron, Oil	GRAS
Estragon, Oil	GRAS
Ethanol	GRAS
p-Ethoxy Benzaldehyde	GRAS
Ethyl Abietate (Purified)	GRAS
Ethyl Acetate	GRAS
Ethyl Acetoacetate	GRAS
Ethyl Aconitate (Mixed Esters)	GRAS
Ethyl Acrylate	GRAS
Ethyl Allyl Acetoacetate	NFA
Ethyl *n*-Amyl Ketone	GRAS
Ethyl Anisate	GRAS
Ethyl Anthranilate	GRAS
Ethyl Benzoate	GRAS
Ethyl Benzoylacetate	GRAS
Ethyl Benzyl Acetoacetate	GRAS
2-Ethyl Butyl Acetate	GRAS
α-Ethyl-*β*-Butyl Acrolein	GRAS
Ethyl Butyl Ketone	GRAS
Ethyl *n*-butyl Malonate	GRAS
2-Ethyl Butyraldehyde	GRAS
Ethyl Butyrate	GRAS
Ethyl *iso*-Butyrate	GRAS
α-Ethyl Butyric acid	GRAS
Ethyl Butyrolactone	Action not completed
Ethyl Carvacrol	NFA
Ethyl Cellulose	NFA
Ethyl Cinnamate	GRAS
Ethyl Crotonate	NFA
Ethyl Cyclohexyl Glycidate	GRAS
Ethyl Cyclohexyl Propionate	FA
Ethyl Decylate	GRAS
Ethyl *iso*-Eugenol	GRAS
Ethyl Formate	GRAS
α-Ethyl Furyl Acrolein	GRAS
Ethyl Furylpropionate	GRAS
Ethyl Guaiacol	FA
Ethyl Heptanoate	GRAS
Ethyl Hexadienoate	GRAS
Ethyl Hexanoate	GRAS
Ethyl Hydrocinnamate	GRAS
Ethyl Lactate	GRAS
Ethyl Laurate	GRAS
Ethyl Levulinate	GRAS
Ethyl *α*-Methylacetoacetate	NFA
Ethyl-*β*-Methyl-*β*-Amyl Glycidate	NFA
Ethyl Methyl Anthranilate	GRAS
Ethyl Methyl *p*-Tolyl Glycidate	GRAS
Ethyl Myristate	GRAS
Ethyl Nitrite	GRAS
2-Ethyl-*γ*-Nonalactone	GRAS
Ethyl Nonanoate	GRAS
Ethyl Octanoate	GRAS
Ethyl Octine Carbonate	GRAS
Ethyl Oleate	GRAS
Ethyl Oxyhydrate	GRAS
Ethyl Phenylacetate	GRAS
Ethyl Phenylbutyrate	GRAS
Ethyl Phenyl Glycidate	GRAS
Ethyl-*α*-Picolinate	NFA
Ethyl Propionate	GRAS
Ethyl Pyruvate	GRAS
Ethyl Salicylate	GRAS
Ethyl Stearate	GRAS
Ethyl Tiglate	GRAS
Ethyl *p*-Tolyl Glycidate	GRAS
Ethyl Undecylenate	GRAS
Ethyl *n*-Valerate	GRAS
Ethyl *iso*-Valerate	GRAS
Ethyl Vanillin	GRAS
Ethylene Glycol Monobutyl Ether Acetate	GRAS
Ethylene Glycol Monomethyl Ether	NFA
Eucalyptol } Eucalyptus Oil	GRAS
Eugenol	GRAS
iso-Eugenol	GRAS
Eugenol Acetate	GRAS
Eugenol *iso*-Amyl Ether	NFA
Eugenol Benzoate	GRAS
Eugenyl Formate	GRAS
iso-Eugenol Formate	GRAS
iso-Eugenol Phenylacetate	GRAS
iso-Eugenylamyl Ether	NFA
Exaltolide	GRAS
Farnesol	GRAS
d-Fenchone	FA
Fenchyl Alcohol	FA
Fennel, Common	GRAS
Fennel, Sweet	GRAS
Fennel, Sweet, Oil	GRAS
Fenugreek	GRAS
Fenugreek, Extract	GRAS
Fenugreek, Oleoresin	GRAS
Fir Needles, Canadian, Oil	GRAS
Fir, Siberian, Oil	NFA
Formic Acid	GRAS
Fumaric Acid	GRAS
Furfural	GRAS
Furfuryl Acetate	GRAS
Furfuryl Alcohol	GRAS
Furfuryl Mercaptan	GRAS
Furfurylidene Acetone	FA
Furyl Acetone	FA
Furyl Acrolein	FA
Fusel, Oil, Refined	GRAS
Galangal, Root	GRAS
Galangal, Root Extract	GRAS
Galangal, Root, Oil	GRAS
Galbanum, Oil	GRAS
Galbanum, Resin	GRAS
Garlic	Food
Garlic, Oil	GRAS
Genet, Absolute	GRAS
Genet, Extract	GRAS
Gentian Root, Extract	GRAS
Geraniol	GRAS
Geranium, Rose, Oil	GRAS
Geranyl Acetate	GRAS
Geranyl Acetoacetate	GRAS
Geranyl Benzoate	GRAS
Geranyl *n*-Butyrate	GRAS
Geranyl *iso*-Butyrate	GRAS
Geranyl Caproate	GRAS
Geranyl Ethyl Ether	NFA
Geranyl Formate	GRAS
Geranyl Phenylacetate	GRAS
Geranyl Propionate	GRAS
Geranyl *iso*-Valerate	GRAS
Germander Herb	NFA
Germander Herb, Extract	NFA
Germander Herb, Extract, Solid	NFA
Ghatti, Gum	GRAS
Ginger	GRAS
Ginger, Extract	GRAS
Ginger, Oil	GRAS
Ginger, Oleoresin	GRAS
Glucose Pentaacetate	FA
Glycerol	GRAS
Glycerol Tributyrate	GRAS
Glyceryl Monooleate	GRAS
Glyceryl Monostearate	GRAS
Glycol Brassylate	GRAS
Glycolic Acid	NFA
Glycyrrhizin, Ammoniated	GRAS
Grains of Paradise	GRAS
Grapefruit, Oil	GRAS
Guaiac Gum, Extract	GRAS
Guaiac Wood, Extract	GRAS
Guaiac Wood, Oil	GRAS
Guaiacol	GRAS
Guaiacyl Phenylacetate	GRAS
Guaiol Acetate	GRAS
Guaiol Butyrate	FA
Guaiol Formate	FA
Guar, Gum	GRAS
Guarana, Gum	GRAS
Guava, Extract	Food
Haw Bark, Black, Extract	GRAS
Hay, Oil	GRAS
γ-Heptalactone	GRAS
n-Heptaldehyde	GRAS
n-Heptaldehyde-2,3-Butylene Glycol Acetal	NFA
Heptanal Glyceryl Acetal	GRAS
Heptal Dimethyl Acetal	GRAS
Heptanone-2	GRAS
Heptyl Acetate	GRAS
n-Heptyl Alcohol	GRAS
Heptyl *n*-Butyrate	GRAS
Heptyl *iso*-Butyrate	GRAS
Heptyl Cinnamate	GRAS
Heptyl Formate	GRAS
Heptyl Octanoate	GRAS
Hexadecanoic Acid	GRAS
n-Hexanal	GRAS
2,3-Hexanedione	GRAS
Hexanoic Acid	GRAS
n-Hexanol	GRAS
2-Hexenal	GRAS
2-Hexen-1-ol	GRAS
3-Hexen-1-ol	GRAS
2-Hexenyl Acetate	GRAS
Hexyl Acetate	GRAS
Hexyl Butyrate	GRAS
Hexyl Caproate	GRAS
α-Hexyl Cinnamic Aldehyde	GRAS
Hexyl Formate	GRAS
Hexyl Furoate	GRAS
Hexyl Octanoate	GRAS
Hexyl Propionate	GRAS
2-Hexylidene Cyclopentanone	NFA
Hickory Bark, Extract	GRAS
Ho, Oil	GRAS
Hoarhound, Extract	GRAS
Holy Thistle Extract	NFA
Hops, Extract	GRAS
Hops, Oil	GRAS
Hops, Extract, Solid	GRAS
Horsemint Leaves, Extract	GRAS
Horseradish	Food
Hydratropyl Butyrate	GRAS
Hydrocinnamic Acid	GRAS
p-Hydroxybenzylacetone	GRAS
Hydroxycitronellal	GRAS
Hydroxycitronellal Diethyl Acetal	GRAS
Hydroxycitronellal Dimethyl Acetal (Schiff Base or Condensation Product of) Hydroxy-Citronellal and Methyl-Anthranilate	GRAS
Hydroxycitronellol	GRAS
ω-Hydroxy-6-Hexadecenoic Acid Lactone	GRAS

3-Hydroxy-2-Methyl-γ-Pyrone	GRAS
1-Hydroxynonan-3-one Acetate	GRAS
Hyssop	GRAS
Hyssop, Extract	GRAS
Hyssop, Oil	GRAS
Immortelle, Extract	GRAS
Indole	GRAS
α-Ionone	GRAS
β-Ionone	GRAS
Irish Moss, Extract	GRAS
α-Irone	GRAS
Isopropyl Acetate	GRAS
p-Isopropyl Acetophenone	GRAS
Isopropyl Alcohol	GRAS
Isopropyl Benzoate	GRAS
Isopropyl n-Butyrate	GRAS
Isopropyl *iso*-Butyrate	GRAS
Isopropyl Caproate	GRAS
Isopropyl Cinnamate	GRAS
Isopropyl Formate	GRAS
β-Isopropyl Phenyl-acetaldehyde	GRAS
Isopropyl Phenylacetate	GRAS
Isopropyl Propionate	GRAS
Isopropyl Quinoline	NFA
Isopropyl *iso*-Valerate	GRAS
Iva Herb Extract	NFA
Ivy Herb (Germander)	NFA
Ivy Herb (Germander), Extract	NFA
Ivy Herb (Germander) Extract, Solid	NFA
Jasmine, Absolute	GRAS
Jasmine, Concrete	GRAS
Jasmine, Oil	GRAS
Jasmine Spiritus	GRAS
Juniper Berries	GRAS
Juniper, Extract	GRAS
Juniper, Oil	GRAS
Karaya, Gum	GRAS
Kelp	GRAS
Kinos, Gum	GRAS
Kola Nut, Extract	GRAS
Labdanum, Absolute	GRAS
Labdanum, Oil	GRAS
Labdanum, Oleoresin	GRAS
Lactic Acid	GRAS
Laurel Berries	NFA
Laurel, Leaves, Extract	GRAS
Lauric Acid	GRAS
Lauryl Acetate	GRAS
Lavandin, Acetylated, Terpeneless	GRAS
Lavandin, Oil	GRAS
Lavender	GRAS
Lavender, Absolute	GRAS
Lavender, Concrete	GRAS
Lavender, Oil	GRAS
Lemon, Extract	GRAS
Lemon, Oil	GRAS
Lemon, Oil, Terpeneless	GRAS
Lemon-Grass, Oil	GRAS
Levulinic Acid	GRAS
Licorice, Extract	GRAS
Licorice, Extract, Powder	GRAS
Licorice, Root	GRAS
Lime, Oil	GRAS
Limes, Oil, Terpeneless	GRAS
d-Limonene	GRAS
Linaloe Wood, Oil	GRAS
Linalool	GRAS
Linalyl Acetate	GRAS
Linalyl Anthranilate	GRAS
Linalyl Benzoate	GRAS
Linalyl Butyrate	GRAS
Linalyl *iso*-Butyrate	GRAS
Linalyl Caproate	GRAS
Linalyl Caprylate	GRAS
Linalyl Cinnamate	GRAS
Linalyl Formate	GRAS
Linalyl Propionate	GRAS
Linalyl *iso*-Valerate	GRAS
Linden Flowers	GRAS
Locust, Gum	GRAS
Logwood Chip, Extract	GRAS
Lovage	GRAS
Lovage, Extract	GRAS
Lovage, Oil	GRAS
Mace	GRAS
Mace, Oil	GRAS
Mace, Oleoresin	GRAS
Maleic Acid	GRAS
Malic Acid	GRAS
Malonic Acid	GRAS
Malt, Extract	Food
Mandarin, Oil	GRAS
Manna	GRAS
Maple, Extract	Food
Marigold, Pot	NFA
Marjoram, Oleoresin	GRAS
Marjoram, Pot	NFA
Marjoram, Seed	GRAS
Marjoram, Sweet	GRAS
Marjoram, Sweet, Oil	GRAS
Mate, Extract	Food
Melilot Herb	FA
Melissa, Oil	GRAS
Melonal	GRAS
1,8-p-Menthadien-7-ol	NFA
Menthol	GRAS
(*d*)-*neo*-Menthol	GRAS
Menthone	GRAS
Menthyl Acetate	GRAS
Menthyl *iso*-Valerate	GRAS
Methallyl Butyrate	NFA
Methoxy-4-*iso*-Hexenyl Tetrahydro Benzaldehyde	Action not completed
o-Methoxy Methyl Benzoate	NFA
2-Methoxy-4-Methyl Phenol	GRAS
1-(*p*-Methoxyphenyl) 2-Propanone	FA
4-(*p*-Methoxyphenyl) Butanone-2	FA
1-(*p*-Methoxyphenyl) Penten-1-one-3	GRAS
p-Methoxytoluene	GRAS
Methyl Acetate	GRAS
p-Methyl Acetophenone	GRAS
Methyl Anisate	GRAS
Methyl Anthranilate	GRAS
Methyl Benzoate	GRAS
Methyl Benzyl Acetate	GRAS
α-Methyl Benzyl Acetone	NFA
p-Methyl Benzyl Acetone	NFA
α-Methyl Benzyl Alcohol	NFA
Methyl *iso*-Butyl Ketone	NFA
Methyl *ter*-Butyl Phenylacetate	FA
3-Methyl, 5-n-Butyl Tetrahydro 4-Pyranyl Acetate	FA
2-Methyl Butyraldehyde	NFA
Methyl-n-Butyrate	GRAS
Methyl *iso*-Butyrate	GRAS
Methyl Cetine Carbonate	NFA
Methyl Cellulose	GRAS
Methyl Chavicol	GRAS
Methyl Cinnamate	GRAS
α-Methyl Cinnamic Aldehyde	GRAS
3-Methyl Coumarin	FA
4-Methyl Coumarin	FA
6-Methyl Coumarin	GRAS
Methyl *o*-Cresyl Ether	GRAS
Methyl *p*-Cresyl Ether	GRAS
1-Methylcyclopentadione-2,3	GRAS
1-Methyl-1-Cyclopentene-2-ol-3-one Butyrate	Action not completed
1-Methyl-1-Cyclopentene-2-ol-3-one *iso*-Valerate	Action not completed
Methyl Decyne Carbonate	GRAS
Methyl Dihydrobietate	GRAS
6-Methyl Benzodihydropyrone	GRAS
Methyl 3,4-Dimethyl 2-3-Epoxy-4-Hexenoate	GRAS
4-Methyl-7-Ethoxy Benzopyrone	GRAS
Methyl Ethyl Acetaldehyde	GRAS
Methyl Ethyl Ketone	NFA
Methyl Eugenol	GRAS
Methyl *iso*-Eugenol	GRAS
5-Methyl Furfural	GRAS
Methyl Furoate	GRAS
α-Methyl Furyl Acrolein	GRAS
2-Methylheptanoic Acid	GRAS
Methyl Heptenone	GRAS
Methyl Heptine Carbonate	GRAS
Methyl Heptylate	GRAS
Methyl Heptyl Ketone	GRAS
Methyl Hexanoate	GRAS
Methyl-2-Hexenoate	GRAS
Methyl Hexyl Acetaldehyde	NFA
Methyl Hexyl Ketone	GRAS
p-Methyl Hydrotropic Aldehyde	NFA
Methyl Hexyl Ketone	GRAS
p-Methyl Hydrotropic Aldehyde	NFA
Methyl *p*-Hydroxybenzoate	GRAS
Methyl-α-Ionone	GRAS
Methyl-β-Ionone	GRAS
Methyl-γ-Ionone	GRAS
Methyl-δ-Ionone	GRAS
α-Methyl-*iso*-Ionone	GRAS
Methyl Laurate	GRAS
Methyl Mercaptan	GRAS
α-Methyl-*p*-Methoxy Cinnamic Aldehyde	NFA
Methyl 4-Methylpentanoic Acid	GRAS
1-Methyl-4(4-Methyl-3-Pentenyl)-1,2,3,6-Tetrahydrobenzaldehyde	NFA
Methyl-β-Methylthiol-propionate	GRAS
Methyl Myristate	GRAS
Methyl α-Naphthyl Ketone	GRAS
Methyl β-Naphthyl Ketone	GRAS
Methyl Nonyl Acetaldehyde	GRAS
Methyl Nonyl Ketone	GRAS
Methyl Nonylen-2-ate	GRAS
Methyl Octanoate	GRAS
Methyl Octyne Carbonate	GRAS
Methyl Pelargonate	GRAS
2-Methyl Pentanoic Acid	GRAS
p-Methylphenyl-acetaldehyde	GRAS
Methyl Phenylacetate	GRAS
Methyl Phenyl Butanone	NFA
3-Methyl-4-Phenyl-Butene 3-one-2	GRAS
Methyl Phenylbutyrate	GRAS
Methyl Phenyl Glycidate	GRAS
3-Methyl-3-Phenyl Glycidic Acid, Ethyl Ester	GRAS
Methyl Phenyl Methyl Glycidic Acid	FA
Methyl Phenyl Methyl Glycidate	GRAS
Methyl Phenylpropionate	GRAS
Methyl Propionate	GRAS
Methyl n-Propyl Ketone	GRAS
Methyl Pyrazine	FA
N-Methylpyrrole	FA
p-Methyl Quinoline	GRAS
Methyl Salicylate	GRAS
Methyl Styryl Carbinol	GRAS
β-Methylthiolpropionaldehyde	GRAS
2-Methyl-3-Tolyl Propanal (Mixed *o*, *m*, *p*)	NFA
Methyl Undecylenate	GRAS
Methyl Undecyl Ketone	NFA
Methyl n-Valerate	GRAS
Methyl *iso*-Valerate	GRAS
Methyl Vanillyl Ketone	NFA
Methylol Methyl Amyl Ketone	GRAS
Mimosa, Absolute	GRAS
Molasses, Extract	Food
Monosodium Glutamate	GRAS
Mountain Maple, Extract, Solid	GRAS
Mountain Maple, Bark	GRAS
Mountain Maple, Bark, Extract, Solid	GRAS
Mousse Nova Tris	FA
Mug Wort	NFA
Mullein Flowers	GRAS
Musk Ambrette	GRAS
Musk Ketone	GRAS
Musk Tibetine	NFA
Musk Tonquin	GRAS
Musk Xylol	GRAS
Musk Zibata	FA
Mustard, Brown	GRAS
Mustard, Yellow	GRAS
Myrcene	GRAS
Myrrh, Gum	GRAS
Myrrh, Oil	GRAS
β-Naphthyl Anthranilate	GRAS
β-Naphthyl *iso*-Butyl Ether	GRAS
β-Naphthyl Ethyl Ether	GRAS
β-Naphthyl Methyl Ether	GRAS
Naringen, Extract	GRAS
Nerol	GRAS
Neroli Bigarde, Oil	GRAS
Nerolidol	GRAS
Neryl Acetate	GRAS
Neryl Butyrate	GRAS
Neryl *iso*-Butyrate	GRAS
Neryl Formate	GRAS
Neryl Propionate	GRAS
Neryl-*iso*-Valerate	GRAS
Nitrous Oxide	GRAS
Nonanal	GRAS
2,6-Nonadienol	GRAS
Nonanediol 1,3 Acetate (Mixed Esters)	GRAS
n-Nonoic Acid	GRAS
n-Nonyl Acetate	GRAS
n-Nonyl Alcohol	GRAS
γ-Nonyl Lactone	GRAS
Nonyl Octanoate	GRAS
n-Nonyl *iso*-Valerate	GRAS
Nonyl Vanillylamide	NFA
Nutmeg	GRAS
Nutmeg, Oil	GRAS
Oak Chips, Extract	GRAS
Oak Moss, Absolute	GRAS
Oak Moss, Extract	NFA
γ-Octalactone	GRAS
Octanal	GRAS
Octanal Dimethyl Acetal	GRAS
Octanoic Acid	GRAS
1-Octanol	GRAS
2-Octanol	GRAS
3-Octanol	GRAS
n-Octyl Acetate	GRAS
n-Octyl Benzoate	FA
n-Octyl Butyrate	GRAS
n-Octyl *iso*-Butyrate	GRAS
n-Octyl Caprylate	GRAS
n-Octyl Ethyl Amine	FA
n-Octyl Formate	GRAS
n-Octyl Heptylate	GRAS
n-Octyl Phenylacetate	GRAS
n-Octyl Propionate	GRAS
n-Octyl Salicylate	FA
n-Octyl *iso*-Valerate	GRAS
Oleic Acid	GRAS
Olibanum	FA
Olibanum, Extract	FA
Olibanum, Oil	GRAS
Onion, Oil	GRAS
Opopanax, Gum	GRAS
Opopanax, Oil	GRAS
Orange Flowers	GRAS
Orange Blossoms, Absolute	GRAS
Orange, Leaf, Absolute	GRAS
Orange Oil, Distilled	GRAS
Orange Oil, Terpeneless	GRAS
Orange, Peel, Bitter, Oil	GRAS
Orange, Peel, Sweet, Extract	GRAS
Orange, Peel, Sweet, Oil	GRAS
Orange, Peel, Sweet, Oil, Terpeneless	GRAS
Oregano	GRAS
Origanum, Oil (Extractive)	GRAS
Orris, Root, Extract	GRAS
Orris, Concrete, Liquid, Oil	GRAS
Palma Rose, Oil	GRAS
Paprika	GRAS
Paprika, Oleoresin	GRAS
Parsley	GRAS
Parsley, Oil	GRAS
Parsley, Oleoresin	GRAS
Patchouly, Oil	GRAS
Pea, Sweet, Oil	NFA
Peach Kernel Oil	Food
Peanut Stearine	Food
n-Pelargonyl Morpholide	NFA
iso-Pentaldehyde	GRAS
4-Pentenoic Acid	GRAS
Pepper, Black	GRAS
Pepper, Black, Oil	GRAS
Pepper, Black, Oleoresin	GRAS
Pepper, Red	GRAS
Pepper, White	GRAS
Pepper, White, Oil	GRAS
Pepper, White, Oleoresin	GRAS
Peppermint, Leaves	GRAS
Peppermint, Oil	GRAS
Perilla, Oil	NFA
Perillyl Acetate	NFA
Petitgrain, Oil	GRAS
Petitgrain, Lemon, Oil	GRAS
Petitgrain, Mandarin, Oil	GRAS
α-Phellandrene	GRAS
Phenoxyacetic Acid	GRAS
2-Phenoxyethanol	FA
Phenoxyethyl *iso*-Butyrate	GRAS
Phenylacetaldehyde	GRAS
Phenylacetaldehyde-2,3-Butylene Glycol Acetal	GRAS
Phenylacetaldehyde Dimethyl Acetal	GRAS



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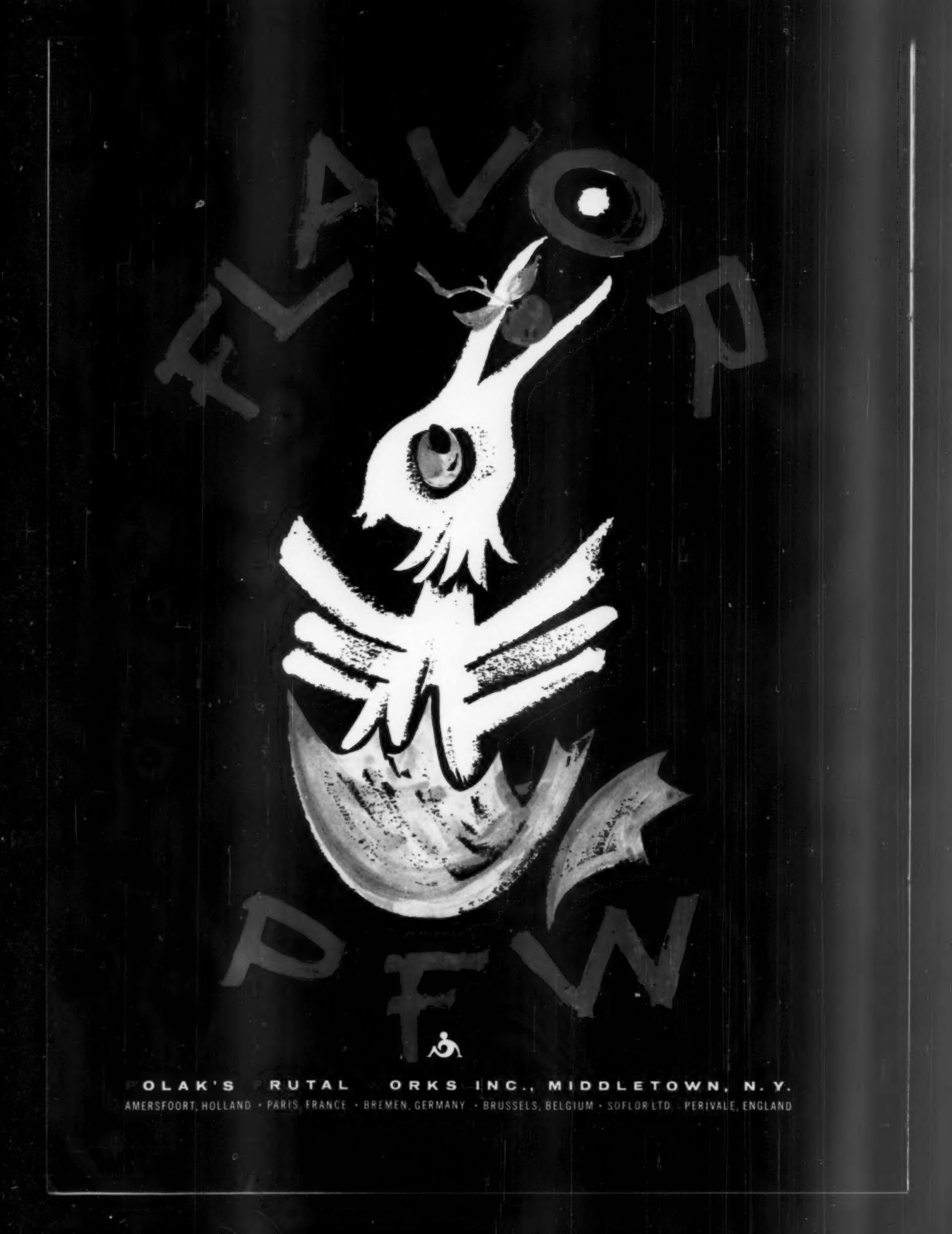

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AMERSFOORT, HOLLAND · PARIS, FRANCE · BREMEN, GERMANY · BRUSSELS, BELGIUM · SOFLOR LTD PERIVALE, ENGLAND

Phenylacetaldehyde Glyceryl Acetal	GRAS
Phenylacetic Acid	GRAS
Phenyl Dimethyl Carbinyl *iso*-Butyrate	NFA
Phenylethyl Acetate	GRAS
Phenylethyl Alcohol	GRAS
α-Phenylethyl Alcohol	GRAS
Phenylethyl Anthranilate	GRAS
Phenylethyl Benzoate	GRAS
Phenylethyl Butyrate	GRAS
Phenylethyl *iso*-Butyrate	GRAS
Phenyl Ethyl Carbinol	NFA
Phenylethyl Cinnamate	GRAS
Phenylethyl Dimethyl Acrylate	NFA
Phenylethyl Dimethyl Carbinyl *iso*-Butyrate	GRAS
Phenylethyl Formate	GRAS
β-Phenylethyl Furoate	GRAS
Phenylethyl Methyl Carbinol	GRAS
Phenylethyl Methyl Carbinol Acetate	GRAS
Phenylethyl Methyl Ethyl Carbinol	GRAS
Phenylethyl Phenylacetate	GRAS
Phenylethyl Propionate	GRAS
Phenylethyl *n*-Propyl Acetal	GRAS
Phenylethyl Salicylate	GRAS
Phenylethyl Tiglate	GRAS
Phenylethyl *iso*-Valerate	GRAS
Phenyl Methyl Carbinol	GRAS
2-Phenyl-1-Propanol	GRAS
α-Phenylpropionaldehyde	GRAS
α-Phenylpropionaldehyde Dimethyl Acetal	GRAS
β-Phenylpropionaldehyde	GRAS
Phenylpropyl Acetate	GRAS
Phenyl *n*-Propyl Alcohol	GRAS
3-Phenylpropyl Butyrate	FA
α-Phenylpropyl Butyrate	GRAS
Phenylpropyl *iso*-Butyrate	GRAS
Phenylpropyl Caproate	GRAS
Phenylpropyl Cinnamate	GRAS
Phenylpropyl Formate	GRAS
Phenylpropyl Propionate	GRAS
2-Phenylpropyl Tetrahydrofurane	GRAS
Phenylpropyl *iso*-Valerate	GRAS
Phenyl Salicylate	NFA
Phosphoric Acid	GRAS
Phthalide	NFA
Pimenta, Oil	GRAS
Pimenta, Leaf, Oil	GRAS
Pimpinella Root	NFA
Pine Cones, Oil	FA
Pine Needles, Oil	GRAS
Pine Oil Tar	GRAS
Pine, Scotch, Oil	GRAS
α-Pinene	GRAS
β-Pinene	GRAS
Pinus Mugo, Var. Pumilio, Oil	GRAS
Piperidine	GRAS
Piperine	GRAS
d-Piperitone	GRAS
Piperonal	GRAS
Piperonyl Acetate	GRAS
Piperonyl Acetone	GRAS
Piperonyl *iso*-Butyrate	GRAS
Pipsissewa, Leaves, Extract	GRAS
Polycyclic Musk	FA
Polyoxyethylene (20) Sorbitan Monolaurate	GRAS
Polyoxyethylene (20) Sorbitan Monooleate	GRAS
Polyoxyethylene (20) Sorbital Monostearate	GRAS
Pomegranate Bark, Extract	FA
Poppy Seed	GRAS
Potassium Acetate	GRAS
Potassium Sorbate	GRAS
Propanal	GRAS
Propenyl Guaethol	GRAS
Propionaldehyde, Hydrogen Sulfide Reaction Product	NFA
Propionic Acid	GRAS
n-Propyl Acetate	GRAS
n-Propyl Alcohol	GRAS
n-Propyl Anisole	GRAS
n-Propyl Benzoate	GRAS
n-Propyl Butyrate	GRAS
n-Propyl *iso*-Butyrate	GRAS
n-Propyl Caproate	GRAS
n-Propyl Cinnamate	GRAS
n-Propyl Formate	GRAS
Propyl Furoate	GRAS
Propyl Furylacrylate	FA
n-Propyl Gallate	GRAS
n-Propyl Heptylate	GRAS
n-Propyl *p*-Hydroxybenzoate	GRAS
Propyl Mercaptan	NFA
n-Propyl Phenylacetate	GRAS
n-Propyl Propionate	GRAS
n-Propyl *iso*-Valerate	GRAS
Propylene Glycol	GRAS
Propylene Glycol Alginate	GRAS
Propylene Glycol Diacetate	FA
Propylene Glycol Dipropionate	FA
Propylene Glycol Monoacetate	FA
Propylene Glycol Stearate	GRAS
Propylidenephthalide	NFA
iso-Pulegol	GRAS
iso-Pulegol Acetate	GRAS
Pulegone	GRAS
iso-Pulegone	NFA
Pyrazine	NFA
Pyridine	GRAS
Pyroligneous Acid	GRAS
Pyroligneous Acid, Extract	GRAS
Pyrrole	FA
Pyruvic Acid	GRAS
Pyruvic Aldehyde	GRAS
Quassia, Extract	GRAS
Quebracho, Bark, Extract	GRAS
Quince, Seed, Extract	GRAS
Quinine Bisulfate RMUL 100 ppm	GRAS
Quinine, Extract	GRAS
Quinine Sulfate RMUL 100 ppm	GRAS
Quinine Hydrochloride	GRAS
iso-Quinoline	GRAS
Raspberry, Leaves, Dried	NFA
Raspberry Seed Oil	NFA
Rennet, Cheese	Food
Resorcinol Dimethyl Ether	GRAS
Rhatany, Extract	GRAS
Rhodinol	GRAS
Rhodinyl Acetate	GRAS
Rhodinyl Butyrate	GRAS
Rhodinyl *iso*-Butyrate	GRAS
Rhodinyl Formate	GRAS
Rhodinyl Phenylacetate	GRAS
Rhodinyl Propionate	GRAS
Rhodinyl *iso*-Valerate	GRAS
Rhubarb Extract	Food
Rhubarb, Root	NFA
Rose, Absolute	GRAS
Rose Hips, Extract	NFA
Rose, Oil, Bulgarian True Otto	GRAS
Rose Water, Stronger	GRAS
Rosemary	GRAS
Rosemary, Oil	GRAS
Rosin	NFA
Rue	GRAS
Rue, Oil	GRAS
Rye, Solid Extract	Food
Saccharine, Sodium	GRAS
Saffron	GRAS
Saffron, Extract	GRAS
iso-Safrol	NFA
Sage	GRAS
Sage, Oil	GRAS
Sage, Oleoresin	GRAS
Sage, Spanish, Oil	GRAS
Salicylic Aldehyde	GRAS
Sandalwood, E. I., Oil	GRAS
Sandalwood, Red	GRAS
Sandalwood, Yellow	NFA
Sandalwood, Yellow, Extract	NFA
Sandalwood, Yellow, Extract, Solid	NFA
Sandalwood, Yellow, Oil	GRAS
Santalol	GRAS
Santalyl Acetate	GRAS
Santalyl Phenylacetate	GRAS
Saponin	GRAS
Sarsaparilla, Extract	GRAS
Sassafras Bark, Extract	NFA
Sassafras, Leaves	NFA
Savory, Summer	GRAS
Savory, Summer, Oil	GRAS
Savory, Summer, Oleoresin	GRAS
Savory, Winter	GRAS
Savory, Winter, Oil	GRAS
Savory, Winter, Oleoresin	GRAS
Schinus Molle, Oil	GRAS
Sesame	Food
Skatole	GRAS
Sloe Berries	GRAS
Sloe Berries, Extract	GRAS
Sloe Berries, Extract, Solid	NFA
Snakeroot, Canadian, Oil	GRAS
Sodium Acetate	GRAS
Sodium Benzoate	GRAS
Sodium Citrate	GRAS
Sodium Hexametaphosphate	GRAS
Sorbitan Monostearate	GRAS
Sorbitol	GRAS
Soya Bean, Oil	Food
Soy Sauce	Food
Spearmint	GRAS
Spearmint, Extract	GRAS
Spearmint, Oil	GRAS
Spike Lavender, Oil	GRAS
Spikenard Root	NFA
Spruce, Oil	GRAS
Squills, White, Extract	NFA
Stearic Acid	GRAS
γ-Stearolactone	NFA
Styralyl Acetate	GRAS
Styralyl Benzylic Ether	GRAS
Styralyl Butyrate	GRAS
Styralyl Formate	GRAS
Styralyl Propionate	GRAS
Styrax, Extract	GRAS
Styrax, Gum	GRAS
Sucrose Octaacetate	GRAS
Sulphur Dioxide	GRAS
Tagetes, Extract	NFA
Tagetes, Oil	GRAS
Tangerine, Oil	GRAS
Tannic Acid	GRAS
Tansey Flowers	NFA
Tansey Flowers, Extract	NFA
Tansey Flowers, Extract, Solid	NFA
Tansy, Oil	NFA
Tarragon	GRAS
Tartaric Acid	GRAS
Tea, Extract	Food
Terpinene-4-ol	GRAS
α-Terpineol	GRAS
Terpinolene	NFA
Terpinyl Acetate	GRAS
Terpinyl Anthranilate	GRAS
Terpinyl Butyrate	GRAS
Terpinyl *iso*-Butyrate	GRAS
Terpinyl Cinnamate	GRAS
Terpinyl Formate	GRAS
Terpinyl Propionate	GRAS
Terpinyl *iso*-Valerate	GRAS
Tetradecanoic Acid	GRAS
n-Tetradecyl Aldehyde	GRAS
Tetrahydrofurfuryl Acetate	GRAS
Tetrahydrofurfuryl Alcohol	FA
Tetrahydrofurfuryl Butyrate	FA
Tetrahydrofurfuryl Propionate	GRAS
Tetrahydro-*pseudo*-Ionone	GRAS
Tetrahydrolinalool	GRAS
1,2,3,4-Tetrahydro-6-Methylquinoline	FA
3,4,5,6-Tetrahydropyronyl-2-Methyl Acetate	NFA
Tetramethyl-Ethyl-Cyclohexenone (Mixture of isomers)	NFA
Thienyl Mercaptan	GRAS
Thyme	GRAS
Thyme, Oil	GRAS
Thyme, White, Oil	GRAS
Thymol	GRAS
iso-Thymol	GRAS
Tolu, Balsam, Extract	GRAS
Tolu, Balsam, Gum	GRAS
Tolualdehyles (Mixed *o*, *m*, *p*)	GRAS
Tolualdehyde Glyceryl Acetal (Mixed *o*, *m*, *p*)	GRAS
Tolyl Acetate	GRAS
Tonka Beans	FA
Tonka Beans, Extract	FA
Tormentilla Root	NFA
Tragacanth, Gum	GRAS
Triacetin	GRAS
Tricalcium Phosphate	GRAS
Tricarballylic Acid	NFA
Trichlormethyl Phenyl Carbinyl Acetate	NFA
2-Tridecenal	GRAS
Triethyl Citrate	GRAS
Triethyl Orthoformate	NFA
Triethyltrimethylcyclo-Hexenone	NFA
Trifolium Extract	NFA
2,6,6-Trimethyl-bi-Cyclo-(1,1,3) Hepten-2-one-4	NFA
Trimethyl Cyclohexanol	NFA
Trimethyl Cyclohexanyl Salicylate	NFA
Tuberose, Oil	GRAS
Turmeric	GRAS
Turmeric, Extract	GRAS
Turmeric, Oleoresin	GRAS
Turpentine, Gum	NFA
Turpentine, Steam Distilled	GRAS
γ-Undecalactone	GRAS
10-Undecenal	GRAS
Undecenyl Acetate	GRAS
n-Undecyl Alcohol	GRAS
Undecylenic Aldehyde	GRAS
Undecylic Aldehyde	GRAS
Valeraldehyde	GRAS
Valderaldehyde and Hydrogen Sulfide, Reaction Product	NFA
Valerian Root, Extract	GRAS
Valerian Root, Oil	GRAS
n-Valeric Acid	GRAS
iso-Valeric Acid	GRAS
iso-Valeric Aldehyde	GRAS
γ-Valerolactone	GRAS
δ-Valerolactone	NFA
iso-Valerophenone	NFA
Vanilla	GRAS
Vanilla, Extract	GRAS
Vanilla, Oleoresin	GRAS
Vanillin	GRAS
o-Vanillin	GRAS
Vanillylidene Acetone	NFA
Veratraldehyde	GRAS
Vetiver, Extract	NFA
Vetiver, Oil	GRAS
Vetiverol	GRAS
Vetivert Bourbon	GRAS
Vetiveryl Acetate	GRAS
Vetiveryl Formate	NFA
Vetiveryl Propionate	NFA
Vetiveryl *iso*-Valerate	NFA
p-Vinyl Guaiacol	GRAS
Violet Leaves, Absolute	GRAS
Walnut Hull, Extract	GRAS
Walnut Leaves, Extract	GRAS
White Pine Bark	NFA
White Pine Bark, Oil	NFA
White Pine Bark, Extract, Solid	NFA
Winter's Bark	NFA
Wintergreen, Extract	GRAS
Wintergreen, Oil	GRAS
Witch Hazel	GRAS
Woodruff, Sweet, Herb	FA
Wormseed Oil	NFA
Wormwood, Extract RMUL 10 ppm on oil	FA
Wormwood, Oil RMUL 10 ppm	FA
Yarrow, Herb	GRAS
Yellow Dock Root	NFA
Yellow Dock Root, Extract	NFA
Yellow Dock Root, Extract, Solid	NFA
Yerba Santa, Fluid, Extract	GRAS
Ylang Ylang, Oil	GRAS
Yucca Brevifolia, Extract	GRAS
Zedoary	GRAS
Zedoary, Bark, Extract	NFA
Zingerone	GRAS

Problems Relating to the Extractability of Food Container Components

L. E. Schniepp, H. O. Etian and J. M. Jackson
American Can Company, Barrington, Illinois

(Manuscript received June 21, 1960)

FOR MANY YEARS, the food container industry has used organic and inorganic components in the coatings, adhesives and sealants employed in the manufacture of food containers. At the time the Food Additive Amendment of 1958 became a law it was necessary to demonstrate that, under the conditions of their intended use, these materials were in compliance with the Amendment. At the present time, the Food and Drug Administration has cleared the use of a large number of components in the manufacture of containers, on the basis of evidence which shows that there is no critical hazard in the continued use of these materials. In the case of metal can coatings alone, some 240 components have been cleared for food containers, many on the basis of prior sanction, and others on the basis of time extension. In a very few instances, where adequate evidence was not available at the time the law went into effect, the materials have been withdrawn from use, until data to establish their safety, under the conditions of intended use, are available.

We are primarily concerned with the behavior characteristics of organic chemical materials; the plastics, can enamels and sealants. These are composed mainly of high molecular weight polymeric materials, but are sometimes blended with small percentages of other components to improve their performance. Those presently in use have been tested and their safety demonstrated.

The organic chemical industry is very active in developing new materials and in seeking new markets for them. Such materials will be evaluated and used for the development of new and improved food containers. Therefore, a program of evaluation and testing for safety must be developed if progress is to be realized.

There are two ways to demonstrate the safety of materials for use in food packaging. If it can be conclusively demonstrated that no extraction occurs on exposure to food and its associated fluids then the material is not a food additive and the provisions of the Food Law Amendment of 1958 do not apply. If extraction or migration does occur, it must be shown that the extracted substances do not constitute a hazard for the consumer of the food.

The Food and Drug Administration *(5)* recognizes the difficulty of analyzing actual food products for traces of organic packaging materials. Therefore, they have suggested the use of simple, synthetic media for use in conducting extraction studies. Specific procedures based on the use of such media which have been developed by the Research Division of the Continental Can Company *(1)* are accepted as suitable for the evaluation of enamel films by the can making industry and by the Food and Drug Administration. A combination of evidence obtained by such extraction studies and long term feeding tests, conducted by and under the sponsorship of the American Can Company *(2)* resulted in the acceptance of the majority of can enamel materials by the Food and Drug Administration long before the Food Law Amendment of 1958 was enacted. It seems probable that the same laboratory procedures will be effective in establishing the safety of new materials for future use in food containers.

MODE OF ATTACK ON THE PROBLEM

From the standpoint of an organic chemist, it seems desirable to attack the problem in relation to the known and expected properties of the various components of the plastic, the enamel film, the sealant, or the adhesive which is to be examined. Thus, by a process of elimination, effort can be expended on the materials most likely to be extracted and their safety or lack of it established without involvement with the innocuous ingredients with which they may be associated. Therefore, an attempt will be made to classify the materials most commonly used in packaging and to discuss the probabilities of their migration into food products.

Enamels. Can enamels consist mainly of resinous materials which have been applied from solvent solution and then baked in an oven to complete the condensation and cross-linking reactions necessary to give coatings which adhere tightly to the metal surface. The resulting films are insoluble in water and most common organic solvents. The base resins for these coatings are generally referred to as thermosetting resins. The most common types are shown in Table 1.

TABLE 1

Thermosetting resins

Phenol-formaldehyde	Epoxy
Urea-formaldehyde	Alkyd
Melamine-formaldehyde	Polyester
Drying oils	Polyurethane

If the application and baking procedures are properly conducted these materials convert to odorless, tasteless, and highly insoluble component parts of the container. In general, they will be found to give negligible extraction under the conditions of proposed use. Probably the most sensitive empirical test which can be applied to show the inert nature of these materials is to expose them to a food product and then conduct a taste test by a panel of trained taste experts using established statistical techniques. Very

slight off-flavors are more readily detectable than is extractability by the usual analytical procedures.

Thermoplastic materials. In some instances polymeric materials other than these thermoset resins may be used either as coatings or for covering specific areas of a food container to improve its resistance to attack by the container contents. The most common of these are the so-called thermoplastic materials which can be melted by heat and which harden on cooling, but without undergoing the curing reactions which characterize the thermosetting resins. The most common commercially available types of these materials are shown in Table 2.

TABLE 2

Thermoplastic resins

Polyvinyl acetate, butyral, chloride, formal and copolymers.
Poly-ethylene, propylene, isobutylene
Poly-acrylate, methacrylate, acrylonitrile
Polystyrene, polyvinylidine chloride
Cellulose esters and ethers
Polyesters-linear (Mylar)

With the polymeric materials it is frequently desirable to incorporate flexibilizing materials or plasticizers, as well as stabilizers and possibly antioxidants. Here again the resins themselves are usually of such high molecular weight that they show no extractability under the usual conditions of testing. The plasticizers, stabilizers, and antioxidants are a different matter. Since they are small in molecular size their tendency to migrate into the media in contact with the film is sometimes significant. Fortunately, the packaging industry recognized this possibility and as a result many of the more commonly used materials of these types have been examined and found to be safe under the conditions of use in food packaging. However, any new materials of the plasticized resin types would have to be subjected to extraction and analytical procedures—and possibly to feeding studies—before clearance for use in food containers.

Elastomers. A third class of polymeric organic chemical materials must also be considered in connection with the safety of food containers. These are the elastomers or rubbers. Years ago, natural rubber in its various cured and uncured states was the only elastic type of material available. Now there are a variety of rubbers which have been found highly efficient materials in the food packaging industry. The more common types are shown in Table 3.

The elastomeric polymers themselves are high molecular weight materials which show little or no solubility in aqueous solutions. Most of them are already considered to be safe for use in food packaging containers. However, for use as a sealant or adhesive they are compounded or mixed with such things as tackifying resins, antioxidants, pigments, fillers, etc. These are the materials most likely to be extracted when the rubber products are brought in contact with food products. Most efforts have been placed on determining to what extent these compounding ingredients may migrate and their biological effect if they do.

TABLE 3

Elastomeric resins (rubbers)

Natural (Hevea)
Butadiene-styrene (GR-S)
Butadiene-acrylonitrile (GR-N)
Polychloroprene (Neoprene)
Isobutylene-isoprene (Butyl)
Polyisoprene (Synthetic Natural)

These compounded elastomeric materials include the sealing compounds which form the seal between the ends and the body of the metal cans. Tops for glass containers also use rubber-based compounds to maintain a seal between the lid and the glass jar or bottle. In some instances, adhesives for paper, metal or plastic containers may consist of compounded elastomeric resins. In all of these applications, only a narrow band or thin edge is in contact with food.

These then are the principal classes of chemical materials with which the food packaging industry must be concerned. Generally, the high molecular weight polymeric materials are of little or no particular concern. Testing procedures have already shown that they are so insoluble in most common food products that there is essentially no chance of migration into food.

Where it is desirable or necessary to incorporate other materials such as colorants, plasticizers, fillers, pigments, antioxidants or low molecular weight resins into container coatings or sealants, they must be selected with great care. Most pigments and fillers are insoluble and chemically inert, and food grade materials are commercially available. Plasticizers and antioxidants have been carefully screened and acceptable grades may be used within specific limits. The use of colorants is questionable, at least until the situation is clarified in terms of the proposed Color Additives Amendment.

FAT-CONTAINING FOODS PRESENT THE MOST SEVERE PROBLEMS

Of all of the various food products those containing appreciable quantities of fats and oils present the most severe problems with regard to migration of components of containers materials. Fats are much better solvents for many of the low molecular weight components used in packaging materials than any of the aqueous brines or syrups associated with non-fatty foods. For this reason, special care is necessary to avoid the use of plasticizers, antioxidants and low molecular weight resins which have not been cleared as safe in the packaging of fat-containing foods. Fats are, in themselves, very complex mixtures and the detection of small amounts of contaminating substances presents great difficulties. For this reason, the use of heptane, instead of fat emulsions, as a synthetic media for extraction studies has recently been authorized.

Since rosin and similar types of low molecular weight resins are commonly used as tackifying agents for sealants and adhesives it seemed desirable to develop analytical methods for their detection. Although

there is currently no evidence that these resins are hazardous it would be reassuring to show that they do not migrate from adhesively bonded seams into the contents of a container. The worst possible combination of conditions was selected when an attempt was made to develop a method for determining traces of rosins in fats.

The literature describes methods for determining rosin acids in tall oil and in varnish-type coatings, but these are not adaptable to the detection of small amounts. A modified Lieberman-Storch colorimetric procedure described by Swann *(4)* was found to give a positive test at concentrations as low as 100 ppm, but no reliable data at lower levels. This observation confirms that of Marcali *(3)* who found it necessary to develop a new technique for determining the extractability of a rosin derivative from neoprene-resin coatings for paper. He developed a reactive dye which gave a chloroform-soluble rosin salt. The concentration of this salt in chloroform solution can be measured by spectrophotometric procedure. A sample of this dye was obtained from Dr. Marcali and the procedure found to give reproducible results, if interfering acidic components were eliminated from the material to be analyzed. However, it has not been possible to separate all of the interfering substances from either natural or synthetic fat-containing extraction media, and the method is not presently applicable to these materials.

Recently evidence has been presented to the Food and Drug Administration to show that under conditions of ordinary usage there is essentially no exposure of tin can end-sealing compounds to the container contents. Since this is the case there is no serious question of extractability or migration. Extraction studies conducted prior to presentation of this evidence had indicated that these materials were at most only slightly attacked by the synthetic extraction media. Only when acidic media were used did any weight loss occur. This was shown to be caused by solution of part of the harmless alkaline earth oxides which these compounds contained.

It has been possible to show, by extraction tests, that an organic colorant added to a base coat enamel does not migrate through a suitable protective top coat. This was done by first developing a spectrophotometric procedure for the analysis of the colorant in a dilute aqueous solution of the extraction media. It was found that as little as 0.1 ppm could be determined with accuracy. The base coat enamel was then applied to aluminum and baked to a fully cured film. Part of this coated plate was then given a second coating of the protective top coat. Strips of single and double coated plate were exposed to boiling extraction media for a week after which the extracts were analyzed. Whereas, a slight leaching of the colorant had occurred from the singly-coated strips, there was no trace of color when the top coat was used.

CONCLUSIONS

Under present circumstances, it is possible to say that the organic chemical ingredients now in use in the manufacture of food containers either do not contribute additives or the use is in compliance with established regulations. It is believed that the container industry has a sufficiently broad background of experience with various materials and test procedures to handle the problems relating to new materials and to assure continued compliance with the Food Additives Amendment of 1958.

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Biological Screening Techniques for Food Additives

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MANY NEW and unusual materials are being considered for direct food additives to enhance the color, aroma and palatability of packaged foods and for food packaging use. There have been innovations in the last 10 years in the development of packaged foods which have included the use of antioxidants, preservatives, and stabilizers in the food. Similarly, new enamel coatings, plastic films, adhesives and other package components have been developed and employed with food products.

Under the new Food Law Amendment to the Food, Drug and Cosmetic Act, it is necessary to prove that these direct food additives and the possible migrants from the package are safe for their intended use. The purpose of this paper is to describe screening techniques to determine whether the direct food additives or migrants of unknown biological activity may be toxic to animals.

The development of new packages or products is a long, tedious and expensive procedure. If, at the end of the developmental period, it is found that the main materials are either toxic or of unknown biological activity requiring a two-year feeding study before a petition will be granted for their use, the financial loss occasioned by this delay may well offset any profit accruing from the new product. Another financial burden to be added to the cost of developing new materials is the cost of a two-year feeding program, which can run well over $50,000.00. When biological screening studies are started on the materials of unknown biological activity early in the developmental stage, some of this delay may be avoided. If the material appears toxic, the developmental work may be stopped at that time.

The first step in a biological evaluation of packaging materials is one of the extraction procedures described by Schniepp, Etian and Jackson *(2)*. When the level of extractant is known, the amount of the test material to be used in the diet of the experimental animals may be easily calculated. If the material to be evaluated is a direct food additive, such extraction studies, of course, are unnecessary, since the level of the additive in the food is predetermined. If this direct additive is introduced before a processing step, however, some chemical study must be made of the finished product to determine whether this additive has remained chemically unchanged during the processing. The material studied biologically must be in the same chemical state in which it will exist in the finished product and not necessarily in the state in which it was introduced into the food.

EXPERIMENTAL PROCEDURE

The subacute 90-day feeding study on rats is the screening tool most preferred in this laboratory. Careful observation of the animals during the entire test period and evaluation of all numerical data by established statistical procedures form the basis of success with this tool. The most important personnel employed in these biological evaluations are the animal caretakers. They must like animals, treat the animals kindly, and see to it that no sudden loud noises or abrupt movements of cage racks occur. If the rats become nervous and irritable, their reactions to both the test and control diets will be altered, and it will be difficult to draw valid conclusions from the data obtained.

When weanling rats, 30 days of age, are placed on a 90-day test, all the control animals should survive the test. There may be an occasional death in this group because of respiratory infection, but three or more deaths in the control group indicate either a poor stock rat or a poor animal caretaker. If there are a number of deaths in the control group, the statistical analysis of the data will not be as sensitive because of the decrease in "degrees of freedom."

Weanling rats, 30 days of age, are caged individually and allowed access to food and water *ad libitum*. There are 10 male and 10 female rats for each test variable and for the control group. The rats are weighed weekly and food consumptions recorded twice a week. Any abnormalities in water intake, food consumption or appearance of the animal and/or the excreta are recorded by the animal caretaker.

The test materials are mixed directly into the dry diet if at all possible. The dry stock ration is prepared twice weekly.

A substitution of more than 10% of a diet by a non-nutritive test material may upset the complete metabolism of the animal. Little faith can be placed in data obtained under such abnormal conditions. If such levels are necessary to reach a 100-fold safety factor, other means of testing the material should be devised. A different method was used in the feeding study on can enamels *(1)*. The canned food was exposed to 10 times the normal area of enameled surface for 90 days at 100° F; this exposed food was then incorporated into the rat's diet at 10 times the level of canned food in the human diet as calculated from USDA statistics. Had the baked enamel film been detached from the can wall and incorporated in the dry diet at 100 times the level of exposure to canned foods, the rat would have been fed ion-exchange resins at amounts which would have upset the physiology of the digestive tract.

If the material is labile on storage or liquid so that incorporation in the dry ration is not feasible, it may be pureed with peas, tomato juice, milk or meat and fed daily in auxiliary dishes. Rats will readily consume quantities of these foods up to 25 g without influencing their intake of the stock diet. These "human foods" have no adverse effect on the health or longevity of the rats *(1)*.

The main constituents of the stock ration are natural grains and milk powder mixed in the laboratory. This diet has been used successfully by us for 25 years. It allows simple incorporation of test materials because it is not pelleted, and it is readily consumed by both rats and mice. The composition of the stock diet is shown in Table 1.

Complete blood studies, made on all animals at least three times during the test period, consist of hemoglobin determination, red and white blood cell counts and a differential white blood cell count. These studies are usually made on the young rats when received in the laboratory and at the mid-point and termination of the test. Drawing blood from the tail of the rat for these tests requires some skill and a lot of patience, and practice is the only solution to the problem.

In this laboratory another blood test is being used experimentally which may give additional pertinent information. This is a determination of the fragility of the red blood cells.

TABLE 1

Stock ration for rats and mice

	%
Whole milk powder	30
Ground yellow corn	30
Ground whole wheat	30
Linseed oil meal (not extracted)	3
Alfalfa leaf meal	3
Defatted whole liver powder	2
Cod liver oil	1
Bone meal	0.5
Iodized salt	0.5

There are indications in the literature that diet will affect the hemolysis and subsequent release of hemoglobin by these cells. However, further investigation of this technique is needed before it can be recommended for routine use in a testing program.

After the final blood studies have been made at the end of the test period, the surviving animals are necropsied. Each animal is carefully examined and the heart, lungs, liver, kidneys, spleen and testes are dissected and weighed. Any tissue which appears abnormal is preserved in formaldehyde for tissue sectioning and microscopic examination. In this subacute test it is not our practice to examine all tissue microscopically. Any tumors or cysts are preserved for histological examination. The urinary bladder is examined for stones and the thyroids are examined for gross enlargement.

RESULTS AND INTERPRETATION OF DATA

An analysis of co-variance is run of the weight gain and food consumption data. This test will differentiate whether any statistical differences in weight gain or food consumption as shown by an analysis of variance are caused merely by differences in appetite, or are due to metabolic effects of the test diet. Table 2

TABLE 2

Weight gains and food consumption of Sprague-Dawley rats fed four test diets and control diet for 90 days

Lots	Average weight gains [1]		Food consumption [1]		Adjusted mean weight gains [2]	
	Males	Females	Males	Females	Males	Females
	g	g	g	g	g	g
122	93.7**	84.8**	991.6**	942.8**	93.7**	84.8**
124	324	169.3	1479.3	1211.1	324	169.4
125	345.7	172.8	1468.6	1093.2*	345.9	172.8
126	330.8	163.5	1450.6	1052.1**	330.8	163.4
127 (Control)	332.5	175.7	1480.9	1190.9	332.6	175.8

* Significant at the 95% level of confidence.
** Significant at the 99% level of confidence.
[1] Analysis of variance test applied.
[2] Analysis of co-variance test applied.

shows some data which illustrate this point. The adjusted mean weight gains of the last column are calculated on the basis of an equal food intake for each group. If statistical differences in these adjusted means are shown by the co-variance test, it means that the diets fed are metabolized differently from the control diet. In this particular case the adjusted mean weight gains differ only slightly from the average weight gains and the same lot (122) is shown to differ very significantly from the controls in both tests. The significant differences in food consumption found in the females of lots 125 and 126 did not affect the adjusted mean weight gains.

In Table 3, however, data are shown in which the

TABLE 3

Weight gains and food consumption of white rats fed ten test diets and two control diets for 90 days

Lots	Average weight gains [1]		Food consumption [1]		Adjusted mean weight gains [2]	
	Males	Females	Males	Females	Males	Females
	g	g	g	g	g	g
110	317	155.3	1691	1248	315.9	158.5
111	301.8	155.4	1703	1251	298.4	158.3
112	302.3	154	1650	1271	309.3	154.3
113	287.9	133.4**	1709	1263	283.4**	135**
114	303.7	153.3	1728	1310	295.4*	150
115 (Control)	306.8	161.3	1633	1329	317.1	156
116	336	165.3	1647	1278	334	165
117	333.5	167.6	1716	1273	321.1	167.4
118	316.3	174.3	1640	1310	315.4	173.6
119	247.8**	127.6**	1487	1172**	270.5**	128.9**
120	329.6	158.3	1685	1264	321.8	158.2*
121 (Control)	310.4	171.9	1630	1264	311.1	171.8

* Significant at the 95% level of confidence.
** Significant at the 99% level of confidence.
[1] Analysis of variance test applied.
[2] Analysis of co-variance test applied.

adjusted mean gains do differ from the average weight gains. In the first group of experimental lots, the males from lots 113 and 114 are shown to have adjusted mean weight gains significantly lower than the controls. This difference was not apparent when only the average weight gains were studied. The females of lot 113 were shown to have significantly lower weight gains by both statistical tests. In the second group of data, the females of lot 120 are shown to have a significantly lower weight gain than the controls although the absolute differences between the two sets of data are equal. This has occurred because the Analysis of Co-Variance tests different information than the Analysis of Variance. According to the calculations described in Snedecor *(4)*, 53% more female rats would have been required in this experiment to show this difference by a straight analysis of variance.

The hemoglobin and red blood cell counts of the rats are indication of the health of the animal and are followed, as explained earlier, during the course of

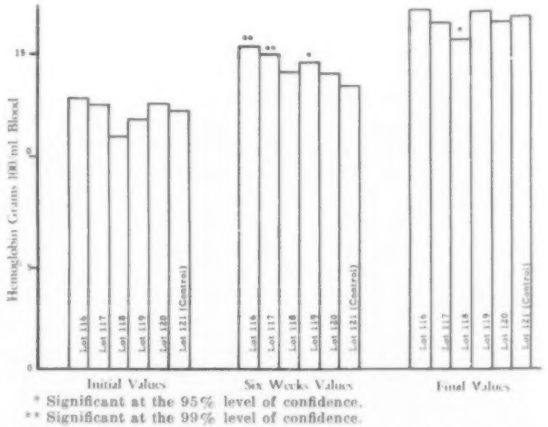

Figure 1. Initial, intermediate and final hemoglobin values of white rats fed test diets compared to the control diet.

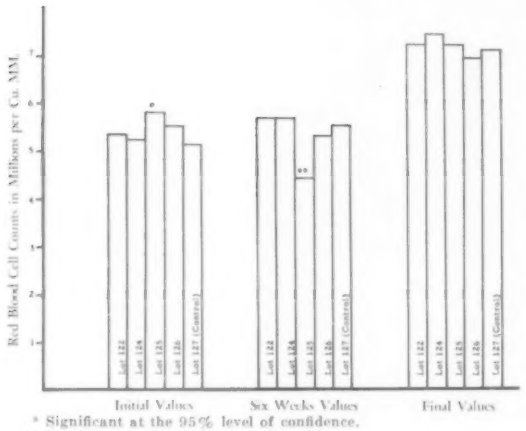

Figure 2. Initial, intermediate and final red blood cell counts of white rats fed test diets compared to the control diet.

the experiment. Figure 1 shows a graphic comparison of the hemoglobin values of one set of experimental animals during the 90-day test period. It may be seen at the 6 weeks examination that while 3 lots had hemoglobin values significantly higher than the controls, all lots were higher numerically than the controls. At the final examination these differences had disappeared and the value of the control lot had increased 3 g per 100 ml of blood. However, lot 118 showed an increase of only 1½ g per 100 ml of blood and was now significantly lower than the controls.

In the routine screening tests of materials in this laboratory, hemoglobin and red blood cell effects are rather rare. This was the only depressed hemoglobin value obtained in the 14 materials discussed in this paper and no significant differences were noted in the final red blood cell counts. In Figure 2 a graphic comparison of the red blood cell counts is shown. The values initially and at the 6 weeks interval for lot 125 differed from the controls, but this difference was not apparent at the final examination. Such variations in hemoglobin and red cell levels are common among laboratory rats and should be acclaimed only with caution.

Differential white blood cell counts are made initially and at the final examination. It is normal for the rat to have a high lymphocyte count of 70% or more and a lower polymorphonuclear neutrophil count of 10 to 20%. If this ratio is reversed or decreased to around one, there has been a toxic effect on these cells caused by the diet. In addition, special care should be exercised to note any pathological forms of the white cells and count the number of nucleated red blood cells. The appearance of these cells in the blood stream of mature rats indicates that a stress has been placed on the blood-forming organs of the body by the diet. A higher percentage (8 or above) of eosinophils may indicate an infection or perhaps an allergy in the animal.

The organ weights of the animals determined at necropsy are divided by their respective final body weight to obtain the ratio of organ weights expressed as per cent of final body weight. It has been shown by Smythe, Weil and Adams *(3)* that organ weights significantly heavier in test animals than the controls are an indication of unusual cellular activity in the organ and probable future micropathology if the experiment were continued.

Not all toxicologists subscribe to this theory; some feel that if the test animals do not gain as much weight as the controls during the experimental period because of food refusal, their organ weight-body weight ratio will be high because of a low body weight. This is not necessarily true, as evidenced by the data in Table 4. The animals in lot 122 were greatly depressed in growth, yet only the heart and testes weights of the males were significantly higher than the controls as determined by an analysis of variance. No significant differences were noted for the females.

Data on control rats from the Sprague-Dawley strain were collected from 8 years of work in this laboratory. Data on the final body weight and organ weights of 194 males and 200 females were tabulated. The final body weight of the males ranged from 250 to 500 g, depending on food intake. By the use of the Bendix G-15 computer, it was feasible to determine various correlation coefficients for these data. The weights of the liver, heart, kidneys and spleen were correlated with the body weight; also the ratios of these organs weights to the body weights were correlated with the body weight.

In seeking a measure which will be as much as possible unaffected by body weight, a lower degree of correlation is actually desirable between this measure and body weight.

Lower correlations between the body weights and organ weights expressed as per cent of body weight indicates that the body weight has the lesser influence on these figures.

TABLE 4

Comparison of weight gain and organ weight-body weight ratios of Sprague Dawley rats fed four test diets and a control diet for 90 days

Lots	Av. body weight		Organ weights expressed as per cent body weight								
			Liver		Kidneys		Heart		Spleen		Testes
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
	g	g	%	%	%	%	%	%	%	%	%
122	169**	162**	4.50	4.36	0.74	0.76	0.36**	0.39	0.17	0.21	1.38**
124	400	241	4.10	3.42	0.66	0.69	0.30	0.34	0.17	0.20	0.77
125	424	246	4.01	3.36	0.63	0.68	0.30	0.35	0.17	0.20	0.83
126	408	218*	4.24	4.51	0.70	0.95	0.31	0.48	0.18	0.27	0.84
127 (Control)	411	250	4.05	3.46	0.68	0.72	0.31	0.34	0.17	0.24	0.83

* Significant at the 95% level of confidence.
** Significant at the 99% level of confidence.

TABLE 6

Summary of statistical analyses of data on 14 groups of experimental Sprague-Dawley rats

Lots	Adjusted weight gains		Final hemoglobin		Red blood cell count		% Liver weight		% Kidney weight		% Heart weight		% Spleen weight		% Testes weight
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
110	0	0	0	0	0	0	0	0	0	0	0	0	*	0	0
111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	*	0	*	0	*	0	0	0	0
113	**	**	0	0	0	0	0	0	0	0	*	0	*	*	0
114	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
116	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
118	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0
119	**	**	0	0	0	0	**	*	0	0	0	0	0	0	0
120	0	*	0	0	0	0	*	0	0	0	0	0	0	0	0
122	**	**	0	0	0	0	0	0	0	0	**	0	0	0	**
124	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
126	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 No significant difference at the 95% level of confidence.
* Significant at the 95% level of confidence.
** Significant at the 99% level of confidence.

TABLE 5

Correlation coefficients* for organ weight-body weight data from Sprague-Dawley rats

	Body wt: organ wt		Body wt: % organ wt	
	Males	Females	Males	Females
Liver	0.654	0.761	0.383	0.401
Heart	0.467	0.478	0.370	0.385
Kidney	0.754	0.677	0.380	0.438
Spleen	0.157	0.302	0.448	0.365

* The correlation coefficient varies from 0 in the case of absolutely no correlation between the variables to unity in the case of a perfect linear relationship.

These data are shown in Table 5. From the information shown in the last two tables, it would appear that no broad statement can be made that a group of experimental rats with depressed body weights will have liver, kidney, heart and spleen weights expressed as per cent of final body weight which are statistically heavier than the controls. Other factors than body weight alone enter this picture.

Seventeen groups of 20 rats each have been discussed and data shown for various steps of the biological screening program. It has been emphasized that statistical analyses of all numerical data are necessary for the proper interpretation. A summary is shown in Table 6 of the 14 experimental groups and the number of statistically significant differences which were found between these groups and their respective controls. A glance at this chart shows that lots 112, 113, 119 and 122 require further consideration for use as direct food additives.

If these materials are to be so used, it will be necessary to determine the lowest toxic dose on a 90-day study. The material should then be fed at 100 times the usage level, the lowest toxic level and an intermediate level for 2 years to rats and to a non-rodent species in order to acquire sufficient information on an unknown material to file a petition under the Food Law Amendment. At the time the feeding levels are chosen to determine the lowest toxic dose, it would be wise to repeat the feeding level previously fed. The significant differences obtained might have been caused by extraneous influences. One must never lose sight of the fact that animal experimentation involves many unknown and uncontrollable variables. It is not possible to obtain the precision from a biological assay that is obtainable from a chemical analysis.

In addition, the toxicity information obtained in this screening test applies to the rat and only to the rat. There is also a variation between species. That is the reason more than one species must be used in a two-year feeding study.

SUMMARY

The biological screening method for toxicity described here is useful for eliminating from consideration as food additives materials which are grossly toxic. A material which appears toxic in this test will be toxic at lower levels in a chronic two-year test. It does not follow, however, that a material which is non-toxic in a 90-day subacute test will be non-toxic on a two-year feeding study. If the material passes this screening test and still shows promise from the technological standpoint, a two-year test will be required to obtain information for a petition under the Food Law Amendment.

In the tests used as examples in this paper, only materials of unknown biological activity were considered. Before embarking on any biological testing, it is always advisable to do a literature search to determine what information is already available and plan the experiment from that information.

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Food Colors—Their Status Under the Law*

Arthur T. Schramm

Manager, Field Sales, National Aniline Division, Allied Chemical Corporation

THE FOOD ADDITIVES AMENDMENT of 1958 is, I am sure, all too familiar to most of us. It is not well known, however, that this amendment excludes from its provision "any substance used in accordance with a sanction or approval granted prior to the enactment of this paragraph pursuant to this Act. . . . " Secretary, Arthur S. Flemming, of the Department of Health, Education and Welfare, has recently acknowledged that among the food additives thus excluded are the so-called "coal-tar colors," use of which has been authorized under Section 406 (b) of the Federal Food, Drug and Cosmetic Act of 1938.

Regulations established under Section 406 (b) include, as conditions for addition of a new color to the permitted list, requirements that are similar to those that because necessary in 1958 for approval of food additives. During the past ten years, while Congress has been considering the whole problem of additives in food, the FDA has been issuing orders limiting the use of various certified food colors. Some colors previously listed as "harmless and suitable for use" in food, have by those orders, been limited to external drug and cosmetic application only. Those colors had been listed after presentation of extensive testing data, including lengthy animal feeding studies to evaluate their potential for chronic toxicity and had been produced under specifications which precisely controlled the composition of the colors. However, the Food and Drug Administration redefined the word "harmless" to mean virtually incapable of producing any harm in any quantity under any circumstances. These actions on the part of FDA were based on its contention that these colors are not "harmless" and hence not suitable for use because they produced adverse physiological effects in test animals when fed at very high levels for long periods of time. The quantities involved were far in excess of and admittedly unrelated to amounts ingested by human beings.

After extensive litigation the United States Supreme Court confirmed FDA's view on December 15, 1958, in the case of Flemming v. Florida Citrus Exchange. Neither FDA, nor industry nor independent scientific opinion regarded that absolutist interpretation of the word "harmless" as scientifically sound. Nevertheless, FDA proceeded to delist FD&C Orange Nos. 1 and 2; FD&C Red No. 32; and FD&C Yellow Nos. 1, 3 and 4.

H. R. 7624 AND S. 2197

Realizing that further implementation of the new interpretation would lead to delisting of practically all colors, a result it did not desire, the Department of Health, Education and Welfare, after preliminary exchanges of views with industry, proposed remedial legislation in 1959. As a result H. R. 7624 and S. 2197 were introduced. S. 2197 was passed by the Senate last year. If legislation is not forthcoming in this session of Congress, it is entirely possible that the public will soon be without color that is essential for much of the food, drugs, and cosmetics now in use. Hence, the Certified Color Industry Commitee has worked closely with the Department to expedite this much needed legislation. Initially, our committee supported H. R. 8945 introduced in the House of Representatives by Representative Curtis of Missouri on July 30, 1957 during the 85th Congress. In subsequent meetings between representatives of various industries and government personnel, most of the differences between that bill and the present proposal were resolved.

Our Committee has given careful consideration to both H. R. 7624 and S. 2197. With respect to the Senate bill, we believe that it is well designed to protect the public health and to accomplish the purpose of authorizing use of suitable safe color additives. The recent hardships occasioned by delistings and threats of delisting can be relieved, without in any way endangering the public health, by giving to the Secretary of Health, Education and Welfare, the authority to establish quantitative limitations on such coloring matter pursuant to the terms of S. 2197. Moreover, under this bill the Secretary is given the power to determine whether the color additive "under the conditions of use specified in the regulations, will be safe. . . . " In making that determination the Secretary must consider (1) the probable consumption or other relevant exposure from the additive; (2) the cumulative effect, if any, of such additive in the diet of man or animals; (3) safety factors which, in the opinion of experts, are generally recognized as appropriate for the use of animal experimentation data; and (4) the availability of needed practicable methods of analysis for determining the identity and quantity of (a) the pure dye and intermediates and other impurities contained in such color additive (b) such additive in or on any article of food, drug or cosmetic and (c) any substance formed in or on such article because of the use of such additive. Those provisions give the Secretary broad discretion to investigate the additive proposed, and to determine whether such color is or is not safe. At the same time, S. 2197 is sufficiently flexible to enable the Secretary to keep pace with advancing scientific knowledge in making the determinations of safety. Thus, the Secretary would be enabled to evaluate both new and existing colors in the light of that knowledge and the unworkable "harmless" test under the 1938 Act would be replaced by a useful one based on reason, current scientific knowledge, and appropriate safeguards.

On January 27, 1960 I had the opportunity to testify on behalf of the Certified Color Industry Committee in support of S. 2197, before the House Com-

* Presented at the Twentieth Annual Meeting of the Institute of Food Technologists, San Francisco, California, May 16, 1960.

mittee on Interstate and Foreign Commerce. Many other groups testified before this same Congressional Committee, and it became evident that the main point of contention is the so-called Delaney cancer clause.

The CCIC recommended the elimination from H. R. 7624 of the cancer clause, detailed in Section 706 (b) (5) (B). That provision singles out carcinogenic substances, and readopts, with respect to such substances, the absolute test sought to be remedied by the proposed legislation. S. 2197 does not contain a similar section but gives broad discretion to the Secretary to determine whether a proposed color is safe, and under its terms the Secretary is authorized to take whatever view of carcinogenicity that the stat of scientific knowledge at that time requires. There is no reason, based on either considerations of public health or scientific knowledge, to abandon the grant of discretion to the Secretary in favor of the absolute prohibition.

VIEWPOINT OF THE COLOR INDUSTRY

Of course, it cannot be said that the color industry, by taking this position, favors cancer any more than it can be said that the Senate by passing S. 2197 without a cancer provision, is in favor of cancer. The question is not one of being for or against cancer, but, rather, how best to deal with the matter from the standpoint of legislation. The Senate's deliberate omission of a cancer provision avoids an unnecessary and what we believe to be an unwise, absolute, rigid standard, while at the same time it adequately protects the public health by the broad grant of power it gives to the Secretary.

We of the color industry are understandably concerned about such absolute legislative standards. During the past few years we have seen our business needlessly suffer because of an absolute "harmless per se" statutory requirement that is regarded as scientifically unsound by all concerned; it is administratively undesirable, and unnecessary for the protection of the public health. We have also seen how much time and effort is required to get remedial legislation. It is now almost six years since the first delisting action was taken against colors certified for use in food, and we still do not have remedial legislation. In view of this historical background we are very much disturbed by Secretary Flemming's support of a flat legislative prohibition against the use of color additives that may be found to induce cancer, and by his suggestion that, if in the future, scientific information should be available to demonstrate a safe level of use for a color found to induce cancer, the precise situation would then be laid before Congress for authorizing legislation to permit that particular safe level of use. It is our opinion that this approach is undesirable because:

(1) It freezes the cancer problem to the state of present scientific information without allowing any flexibility for future action in the light of ever increasing knowledge.

(2) It singles out for special and prohibitory treatment an element, cancer potentiality, which is but one of the many complex factors which bear on the basic test of safety, and it really adds nothing to that test.

(3) It would require the Congress to make a scientific judgment in the future. Instead of Congress' setting the broad legislative standard of safety and leaving the implementation of that standard to administrative discretion in the light of evolving scientific knowledge, Congress would be called upon sometime in the future to review scientific information and to determine on the basis of that information whether to remove the proposed prohibition against cancer producing substances. Thus, Congress would be called upon to make a decision that is essentially the business of the Secretary to make.

(4) The Secretary's proposal could well lead to the same situation in which we find ourselves today—that is, all might be agreed that the legislative restriction is no longer necessary and should be removed, but meanwhile, the business of the producers and users of colors might be disrupted without any necessity for doing so in the interest of the public health.

For the foregoing reasons we would prefer omission of any cancer provision from the legislation. We would leave it to the Secretary to deal with carcinogenic manifestations and are confident that his discretion would be employed in the light of the best scientific information available. However, we can understand why, in view of the current widespread concern about cancer, it may be thought desirable to give the Secretary some specific directions in this area. Accordingly, we would not object to a provision giving the Secretary discretionary rather than mandatory authority.

At this point it should be carefully noted that the discussion of the Delaney clause in the present hearings is centered on issues unrelated to the use of colors—issues which instead come under the Food Additives and Pesticide Chemicals Amendments and involve the use of non-color chemicals in animal feeds and problems arising from the recent cranberry and poultry episodes. In other words, the color additive hearings have been used as the battleground for the food additive aspects of the Delaney clause. It should be further noted, that so far as we know, none of the colors now in use is a suspected carcinogen. Hence, we are prepared to accept a Delaney clause now rather than have legislation held up for resolution of problems relating to the use of other forms of additives under other provisions of the law, if that is the only way legislation is to be obtained in this session of Congress.

As you know, Secretary Flemming appeared a second time before the House Committee on May 9, 1960. The hearings are now concluded with five days allowed for the submission of additional comments for the record. It is hoped that Chairman Oren Harris will conduct a series of executive sessions for the full committee to consider this legislation and that the bill be passed by the Congress this session.

With the passage of this legislation, certain administrative problems still pending would be obviated. Among these are the color definition controversy, and

the proposed delisting of seventeen D&C colors commonly referred to as the "lipstick colors."

I would like to point out the status of another administrative problem. As you know, FD&C Orange Nos. 1 and 2, FD&C Red No. 32, and FD&C Yellow Nos. 1, 3 and 4, have been delisted but recently the Food and Drug Administration has issued an order revoking certificates issued on these colors even though such certificates were issued prior to the effective delisting date. In other words, if previously certified lots of these colors are added to food after April 6, 1960, such food would be regarded as adulterated. The Certified Color Industry Committee has filed a petition in the U. S. Court of Appeals for the Second Circuit to set aside FDA's order. It is expected that this petition will be heard soon.

FD&C RED NO 1

In July 1959 an order was issued changing analytical specifications for FD&C Red No. 1. The purpose of this order was twofold: to reflect an improved definition of this color based on modern analytical procedures and, thereby permitting toxicological evaluation of material of a more definite composition. Accordingly, an animal testing program has been initiated on material meeting these new requirements. In October, 1959 FDA published an order declaring that certificates for existing batches of FD&C Red No. 1 that do not comply with the new specifications would cease to be effective January 15, 1960. A subsequent FDA notice published in response to objections filed by the Certified Color Industry Committee and others, postponed the effective date until April 6, 1960 "to allow time for filing petitions for judicial review." FDA contended in this notice that the objections indicated above raise a legal issue that could not be settled by a public hearing. Therefore, on February 9, 1960 our Committee filed a petition with the U. S. Court of Appeals for the Second Circuit requesting that the FDA order be set aside. One of the contentions on which our argument is based is that the purpose of the Federal Food, Drug, and Cosmetic Act of 1938 is to protect public health and the statuatory delegation of authority is intended to achieve that purpose, and that in the case of FD&C Red No. 1, no question has been raised concerning toxicity. By agreement with Government attorneys the effective date of the FD&C Red No. 1 order has been extended from April 6, 1960, until thirty days after final disposition of the Second Circuit proceeding.

Antibiotic Preservation of Beef with Subsequent Feeding to Experimental Animals[a, b]

R. B. Sleeth,[c] J. C. Armstrong,[d] H. S. Goldberg, and H. D. Naumann

Food Processing Laboratories, Missouri Agricultural Station and Department of Microbiology, University of Missouri, Columbia, Missouri

(Manuscript received June 9, 1960)

SINCE THE INTRODUCTION of antibiotics as therapeutic agents, considerable interest has been aroused in the food industry as to their potentials in preserving foods. In the meat branch of the industry considerable interest has been shown in the use of oxytetracycline (OTC) as an ante-mortem injection and post-mortem surface spray for beef to permit accelerated aging at relatively high temperatures *(5)*. By using this procedure, the serious problems of deep seated spoilage and surface microbial growth were substantially reduced. Results have been obtained to show that the quality of lower grade carcasses may be improved as a result of high temperature aging made possible by antibiotics.

Adding an antibiotic to a food brings to focus some of the basic questions with regard to food additives. It is necessary to know, for example, that an antibiotic, like any other additive, will not be a hazard to the consumer. The consumer must be assured that the material added is neither acutely toxic nor toxic on usage over an extended period of time. In addition, the consumer must be assured that general usage of antibiotics in foods will not give rise to large numbers of spoilage or pathogenic organisms that are resistant to antibiotics.

The literature offers some interesting data perti-

[a] Presented before the Twentieth Annual Meeting of the Institute of Food Technologists, San Francisco, California, May 16, 1960.

[b] Journal Series Paper No. 2165, approved by the Director of the Missouri Agricultural Experiment Station.

[c] Present address: Meat Research Section, Food Research Division, Armour and Company, Chicago, Illinois.

[d] Present address: Public Health Department, School of Medicine, Tulane University, New Orleans, Louisiana.

nent to these problems. Goldberg *et al (4)* using chloromycetin, aureomycin, and terramycin on ground beef samples to prolong storage life found that the antibiotics began to disappear within 24 hours and after 72 hours were no longer detectable. Cahill *(1)* added 2 ppm of aureomycin to ground beef and found only a fraction of a ppm remained after 3 days; none remained when samples of infused rounds held at room temperature for 2 days after slaughter were analyzed for residue.

On the basis of several animal studies, Goldberg *et al (3)* indicated that the potential hazard of emergence of antibiotic-resistant bacteria involved little, if any, risk with the use of the tetracycline antibiotics. Further, the over-all percentage of organisms becoming resistant was shown to be limited and the duration of resistance was not permanent.

Carey *(2)*, in a recent review of the results of long term administration of chlortetracycline and other tetracyclines, supported the thesis that the chances of harmful effects to humans from occasional ingestion of small amounts of residues in antibiotic-processed foods are negligible.

But the possibility seems to exist that resistant bacteria, which might be pathogenic to humans, could appear in certain environments subject to the constant presence of antibiotics. This study was therefore undertaken to determine whether beef treated with antibiotics would bring about the development of antibiotic-resistant bacteria.

MATERIALS AND METHODS

Twenty Standard and Utility cattle, weighing 600–700 lb were used in this study. All cattle, except controls, were injected intramuscularly (anterior portion of the tail) with OTC at the rate of 0.80 mg per pound of body weight 30 min to 6 hr ante-mortem.

Detailed antibiotic injection and surface spraying, aging, sampling, OTC assay, and bacteriological procedures are given in an earlier report of this study *(5)*.

OTC feeding of rats. Sixty albino rats of the same inbred strain were used in this study. They were equally divided as to sex and were similar in age and weight (310 to 360 g).

Each rat was caged separately and fed 10 g of fresh, ground meat twice daily supplemented by weekly portions of raw spinach. Fifteen male and 15 female rats were fed cooked meat containing between 0.8 and 1.0 meg of OTC per gram of meat prepared from cattle described above (diet 1). The meat was broiled to an internal temperature of 60° C (140° F) and then ground first through a one-fourth and then a one-eighth inch plate. A similar group of 30 rats was fed raw meat containing between 2.4 and 2.8 meg of OTC per gram (diet 2). The level of OTC was determined by microbiological assay using *Bacillus cereus var mycoides* as the test organism.

Control data were collected from the 60 rats by feeding similar antibiotic-free diets for 2 weeks at the beginning of the experiment.

Examination of gut flora. Fresh fecal specimens were obtained from the rats shortly after the morning feeding in order to take advantage of the gastro-colic reflex. The specimens were pooled in 4 groups (by sex and diet), weighed, and comminuted in quantities of normal saline sufficient to give initial dilutions of 1:100. Serial, hundred-fold tube dilutions of the fecal suspensions were then performed. Aliquots of 0.1 ml from the last 3 tubes in each of the 4 series were pipetted into 8 separate plates of Trypticase Soy Agar (BBL) and spread evenly over the media to give final dilutions of 10^{-6}, 10^{-7}, and 10^{-8}. Oxytetracycline, 10 meg per ml, was added to one-half of the TSA medium before pouring plates in order to measure any resistance to OTC developed by the gut flora of the animals. One-half of the inoculated plates, with and without OTC, were incubated aerobically and one-half were incubated under anaerobic conditions in Brewer jars, all at 37° C. Colonies were counted at the end of 24 and 48 hr. The predominant fecal bacteria were identified merely on the basis of colonial and microscopic morphology, Gram stain reaction, oxygen requirement, and growth rate on TSA medium.

This procedure was carried out 6 times with consecutive daily fecal specimens from all 60 rats to get an estimate of the normal gut flora before meat containing OTC was fed to the animals. The same methods were repeated on pooled specimens collected prior to the weekly sacrificing of 10 rats for tissue assay (see below) and again 6 times on feces from the last group of 10 rats which were held on the test diet for 16 weeks.

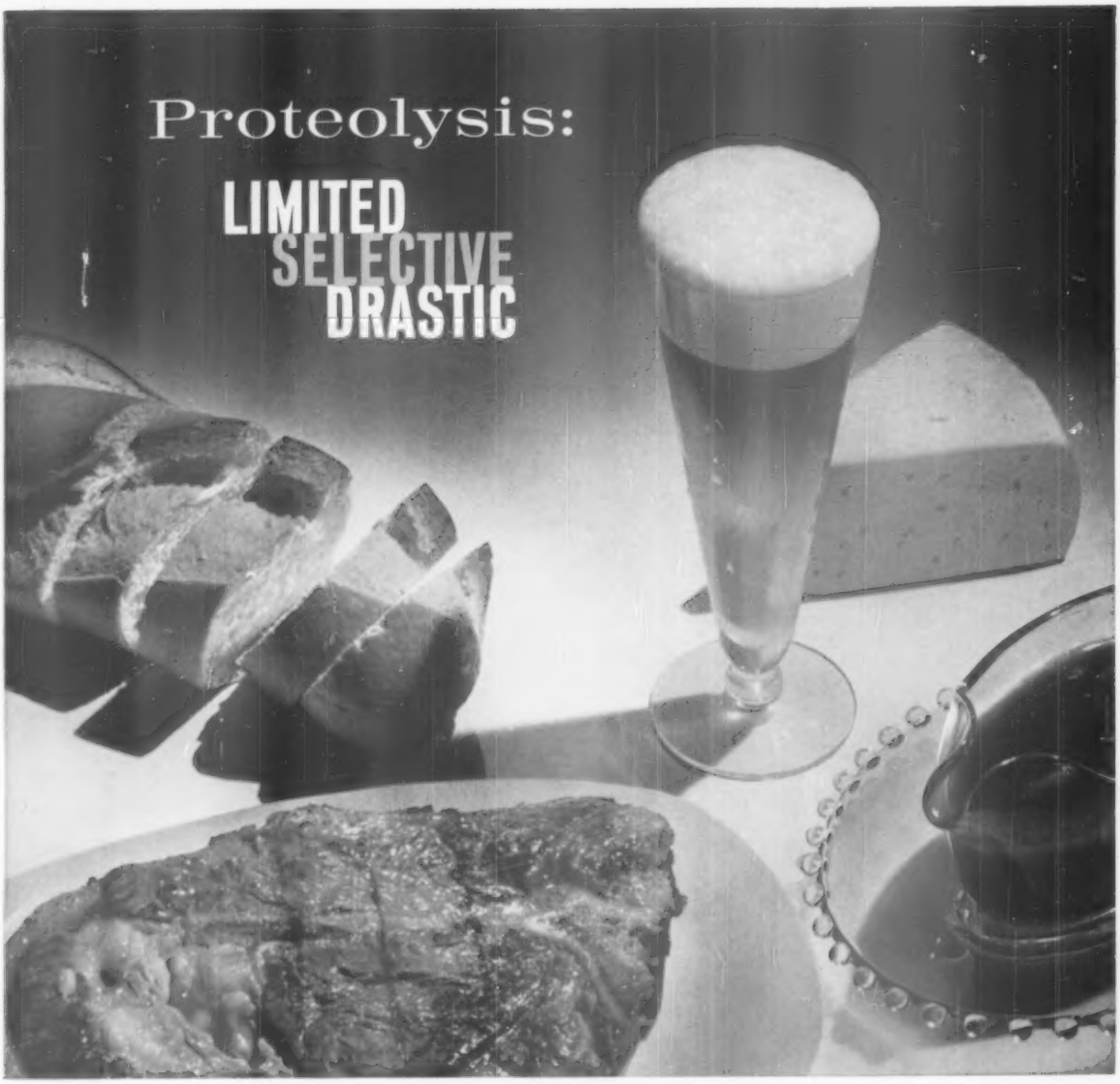
Colony counts. All plates were counted after an incubation period of 24 hr. Plates with greater than 300 colonies were recorded and discarded. The remainder was counted with the aid of a Quebec Colony Counter, re-incubated under the same conditions, and counted again after an additional period of 24 hr. Colony counts for the control data were summarized by computing the arithmetic mean from all countable plates in each category (Table 1). Countable plates were those having between 30 and 300 colonies. The resulting figures were adjusted to a dilution factor of 10^8. The summarized test data (Table 1) were computed in like manner from all of the accumulated plate counts made after the OTC test diets were begun.

Assay of residual OTC. Rats were anesthetized and blood collected by severing the carotid arteries. The serum was decanted from the blood samples after 2 hr at room temperature, centrifuged to remove any cells, and refrigerated. Two to 4

TABLE 1

Summary of plate counts of rat feces before and after low-level feeding with oxytetracycline

Rat group			Colony counts, 10^8 dilution factor							
			Control (diet free of OTC)				Test (diet containing OTC)			
			Aerobic		Anaerobic		Aerobic		Anaerobic	
Sex	Diet		TSA	TSA with OTC	TSA	TSA with OTC	TSA	TSA with OTC	TSA	TSA with OTC
Males (30)	Cooked meat (15)	24 hr count	92	40	37	64	54	22	130	72
		48 hr count	410	150	620	320	140	96	1100	640
	Raw meat (15)	24 hr count	58	60	180	51	120	88	28	2
		48 hr count	180	84	730	450	250	160	430	350
Females (30)	Cooked meat (15)	24 hr count	210	130	200	1	58	36	44	37
		48 hr count	270	190	320	170	190	120	680	280
	Raw meat (15)	24 hr count	78	54	85	40	41	60	76	23
		48 hr count	330	82	1400	380	140	100	470	320



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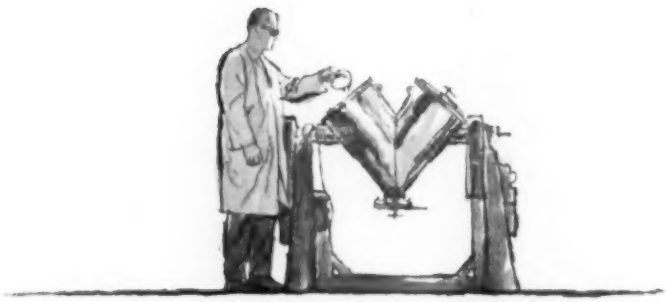

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ml of serum was collected from each rat. Individual muscle and liver samples were obtained from each animal. However, due to size, the 5 heart and kidney samples from each treatment were pooled. These tissues were then assayed for OTC by the procedures previously outlined *(5)*.

RESULTS AND DISCUSSION

The initial phase of this study was designed to determine if the feeding of beef containing low levels of antibiotic to rats would result in the accumulation of residual quantities of OTC in various tissues. Therefore, rats were fed raw and cooked meat containing from 2.4 to 2.8 and from 0.8 to 1.0 meg of OTC per gram, respectively, per day. The feeding of raw and cooked meat with these levels did not cause any detectable ($<.03$ meg/g) residual in blood serum, heart, kidney, or muscle when fed over a period of 16 weeks.

Similarly, the second phase was to ascertain the effect of feeding this beef on the microbiological flora of the rat. A few patterns are apparent in Table 1, which summarizes the plate counts from the OTC feeding experiment. With some exceptions, 48-hr counts on the anaerobically incubated plates were considerably higher than those on duplicate plates incubated aerobically, although 24-hr counts on the same plates were not significantly different. In almost all cases, less growth was obtained on TSA medium containing 10 meg of OTC per ml than on paired plates free of antibiotic. No consistent differences were noted among the plate counts with regard to the sex of the rats or the preparation of their food (cooked or raw). Most importantly, the over-all configuration of the colony counts for the control data was not materially changed after the diets containing OTC were initiated.

Plates inoculated with fecal suspensions diluted to 10^{-5} were not countable in most instances. They were of use mainly in checking media and dilution technics and also in indicating the presence of fast-growing spreaders and swarmers which were rarely cultured from the more highly diluted inocula. Members of the families lactobacillaceae, micrococcaceae and enterobacteriaceae were the predominant bacteria seen at the higher dilutions. Enterococci were probably the most abundant kind cultivated, though lactobacilli and Gram negative rods were also present in great numbers. Micrococci demonstrated a wide diversity of colonial forms and were the dominant type on some plates.

Colony counts were generally lower on plates containing OTC than on paired plates without the antibiotic, but no proportional differences of great magnitude were noted in the kinds of bacteria seen on the two media in this study. However, the relatively few colonies of clostridia that were identified seemed to occur more frequently on plates containing OTC. Otherwise, the predominant forms in the fecal flora appeared to be equally inhibited by OTC. Furthermore, the population proportions of the various broad groups appeared to remain fairly stable throughout the experiment.

An increase in the numbers of *Proteus* might have been anticipated since these common inhabitants of the colon are known to be resistant to OTC. Neither of these organisms, nor any others, were observed to have increased markedly. It is quite likely that some fastidious groups of bacteria, such as the clostridia and *Bacteroides*, were quantitatively underestimated due to the crowding effect of other bacteria, the shortness of the incubation period, and the fact that they were well aerated prior to being plated and again during the 24-hr colony counts.

Under the conditions of this study, it was not possible to detect whether or not degrees of resistance to OTC were developed by the fecal flora. Apparently minor changes may have taken place which, over a longer period of time, would have become bacteriologically or clinically evident. However, the collected data gave the strong impression that no significant changes had occurred in the gut flora following low-level exposure to a broad-spectrum antibiotic for 16 weeks.

SUMMARY AND CONCLUSION

Raw and cooked meat containing low levels of OTC, when fed over a period of 16 weeks, did not cause any detectable residual in blood serum, heart, kidney, or muscle of rats. Apparently, no significant changes occurred in the gut flora of rats following low-level exposure to OTC for 16 weeks. On the basis of this study, the potential health hazard of the consumption of meat containing low levels of OTC would appear to be small.

Acknowledgments

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Nutritional and Chemical Changes Occurring in Heated Fats: A Review

(Manuscript received March 5, 1960)

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THE IMPORTANCE OF FAT in the American diet is evidenced by the simple fact of the amount consumed. Annual per capital consumption of fats in the U.S.A. is close to one-hundred pounds per year. This consists roughly of forty-five pounds of visible fats and fifty-five pounds of invisible fats *(15, 41)*. Visible fat refers to fat consumed as butter, margarine, lard, shortening or vegetable oils; invisible fat refers to fat closely associated with other food components.

The role of fats and their derivatives in health and nutrition has recently become of great interest to the layman, the nutritionist, and the chemist. It is the purpose of this review to summarize the available literature concerning the effects of various physical treatments on fats and to point up the need for further applied research and practical information concerning the processing and use of dietary fats. Certain types of such treatments may possibly result in a loss of nutritional value in the fat. Fats altered in this way, and which may conceivably be produced in the deep fat frying of foods and in other processing operations, are organoleptically acceptable in the human diet but have been shown to exert a growth depressing effect when fed to animals. The occurrence of growth depressing factors in such oils and their physiological and nutritional effects are of concern to all industries dealing with the processing, production, and sale of edible oils and their use in deep fat frying operations.

Most research on heated fats has been concerned with the types of compounds formed during polymerization. These studies have included work on the mode of polymer formation, structure, conditions for optimum polymerization, and the nutritional and physiological effects of polymers. Only a minor amount of applied research, that which actually shows the effects of various processes on polymer formation, has been published and any conclusions of value to industry are probably the result of extrapolation from basic data.

It has been generally recognized that 3 types of degradative changes can occur in a fat or oil. These are: a) autoxidation, b) thermal polymerization, and c) thermal oxidation. For the sake of convenience, autoxidation is defined as the oxidation of a fat or an oil at temperatures up to 100° C; thermal polymerization as taking place between 200–300°, in the absence of oxygen; and thermal oxidation as oxidation at about 200° C in the presence of air or with aeration.

AUTOXIDATION, THERMAL POLYMERIZATION, AND THERMAL OXIDATION

Autoxidation. The effect of heat on the nutritive value of fats has been widely studied. It has been shown that animals fed corn or cottonseed oils oxidized at 100° C for long periods of time developed vitamin A deficiencies and eventually died *(33, 34, 45)*. Kaunitz at Columbia has shown that autoxidized cottonseed oil is nutritionally harmful to rats *(62)*. Efforts have been made by various research groups to link the poor nutritional value of heated fats to the destruction of essential dietary factors. A number of investigators *(48, 49, 51, 68, 81)* have shown that essential fatty acids, carotene, vitamin A and biotin are destroyed during the autoxidation of fats. Kaunitz *(61)* has reported that riboflavin exerted some protective effect on animals fed rancid diets. Irwin *et al* found that factors other than destruction of essential nutrients are involved in the harmful effects of oxidized fats. These authors demonstrated that oxidized fat does not appear to be completely absorbed in the rat *(51)*. Kaunitz and others have proved satisfactorily that polymeric material formed during the autoxidation of fats depresses growth of rats, even in the presence of excess dietary factors *(62)*.

Whipple *(108)* and Barnes *(5)* fed to dogs, at the 20% level, fat that had been autoxidized at 60° C. They noted that the dogs a) declined rapidly in weight, b) exhibited diarrhea with some hemorrhage, c) developed loss of hair, d) skin lesions, e) ulcers, and eventually became weak and died. Such experiments are unrealistic because of the environmental conditions used and the high percentage of fat fed in the diets, but they do show the effects of oxidized oils under extreme conditions.

Thermal polymerization. Nutritional effects of thermally polymerized oils have also been studied. Raju and Rajogopalan and other groups observed that rats fed unsaturated oils which had been heated for prolonged times at 300° C *(2, 3, 4, 87, 89, 91)* exhibited a depression in growth. It is possible in some of these cases that degradation products may account for the toxicities involved. It has been reported that lard and coconut oils when heated to 300° C, form long chain carbonyl compounds which could be toxic when fed to animals *(47)*.

An extensive series of studies by Crampton and others in Canada has answered many of the questions concerning the toxicity of thermally polymerized oils *(25–30, 40)*. This group has shown that unsaturated oils when heated to 250–300° for 6 to 24 hr contain polymeric material coresponding to cyclic-monomeric,

dimeric, trimeric, and higher polymers. Nutritional studies on these oils have shown that the toxicity or growth depressing effects of such oils is caused by polymeric materials formed in the oils. Other physiological effects caused by heat polymerized oils have been reported. Farmer *et al (40)* have reported that a diet containing only 10% by weight of thermally polymerized oils had an adverse effect on reproduction and lactation in the rat.

Roffo *(93–99)* maintained that animal and vegetable fats such as lard, tallow, olive oil, safflower oil and cholesterol heated up to 350° C for only 30 min caused cancerous growths in his test animals. Work by other investigators did not substantiate these findings. Klein and Palmer *(65)* have pointed out that the tumors induced by Roffo failed to satisfy criteria of true cancerous growths, such as irreversibility, metastases, and transplantability. Work done by Lane *(69)*, Peacock *(82, 83)* and Waterman *(107)* indicates that lesions can be produced by feeding animals heated fats. Since it has not yet been proven whether or not these types of treatments cause the formation of materials not produced by deep frying, it appears obvious, in the light of such findings concerning the lesions formed when heated fats are ingested, that much work of a more definitive nature needs to be done before this aspect of the heated fat picture can be either accepted or disregarded.

It is of interest to note that Morris *(76)* has observed that diets containing 50% of lard heated for only 2 hr at 300° C caused a vitamin E deficiency and gastric ulcers when fed to rats. Alfin-Slater *(1)* has recently shown that unsaturated oils heated at about the same temperature for 1 to 3 hr, until an iodine value drop of 10% occurred, also caused vitamin E deficiency when fed to animals at the 15% level. According to Alfin-Slater's data, this deficiency was corrected by vitamin E supplementation. No evidence of similar undesirable effects could be found when unsaturated oils, heated in this way with an iodine value drop of 5%, were fed to rats at the same level.

The nutritional value of oils aerated at frying temperatures has not been extensively studied. The majority of this work has been done on unfractionated oil samples. Johnson *et al* found that corn oil oxidized at 200° C for 24 hr exhibits a definite growth depressing activity. They also reported that thermally oxidized hydrogenated vegetable oil and butter oil caused very little growth depression *(53, 56, 110)*. Other investigators *(55)* have indicated that oils thermally oxidized at 200° C are not completely absorbed in the intestinal tract of the rat,. Enlarged livers were also noted and evidence relating the amount of polymeric material present in these oils to their nutritional value was presented. It was noted that, as the percentage of polymer increased, nutritive value decreased.

In a recent report, Dangoumau and Associates *(31)* attempted to duplicate the growth depressing effects of oils thermally oxidized at 200° C. Thermally oxidized corn, cottonseed, and peanut oils were fed to rats at the 20% level along with cellulose. Male rats fed thermally oxidized peanut oil for 60 weeks showed growth depression, but female rats under the same conditions showed little depression. Identical results were obtained when male and female rats were fed oxidized cottonseed oil for 20 weeks. When both male and female rats were fed oxidized corn oil for 20 weeks no growth depression was observed. No serious pathological changes were noted.

The nutritional effects of polymeric fractions isolated from thermally oxidized corn oil have also been investigated *(84)*. Polymeric fractions so obtained were fed to male weanling rats which had been maintained on a complete diet for 21 days. Water and oil soluble vitamins were also provided. Weanling rats fed the non-distillable residue from the non-urea adduct-forming fatty acids of thermally oxidized corn oil at a 12% level all died within 5 days. These animals lost an average of 7 g while those on the fatty acids of fresh oil acids gained 16 g on the same amount of food intake during the 5-day period. Dilution of the non-urea adduct forming acids (NAFA) with an equal amount of fatty acids from fresh corn oil resulted in survival of the animals for the 21-day test period, but only partially counteracted the growth depressing effect of the non-adduct forming acids. Animals receiving this mixture had gained only 15 g while those on the fatty acids from fresh corn oil gained 85 g during the 21-day test period. Although fatty acids from thermally oxidized oil depressed growth significantly, rats fed the urea adducting material, which represented 64% of the oil, gained as much weight as rats fed the fatty acids from fresh corn oil. Furthermore, the molecular distillate of the NAFA, which represented 15% of this fraction, did not depress growth significantly. It was also shown that the portion of non-urea adduct-forming material in the treated oil and the molecular weight of the polymeric materials *(84, 55)*, seem to be a better index of nutritive value of heated fats (Figures 1 and 2) than iodine values.

Few reports have appeared concerning the effects of feeding oils found in commercial usage *(110)*. In one study a) corn oil samples drawn from a batch type and a continuous type potato chip fryer, b) lard drawn from a batch type french fryer in a restaurant, and c) a hydrogenated vegetable oil sample drawn from a commercial doughnut fryer were fed to rats

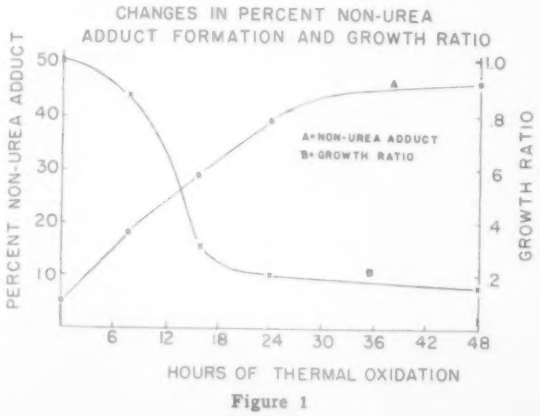

Figure 1

Figure 2. Comparison of constants and biological response to various fractions of the fatty acids from fresh and thermally oxidized corn oil.[1]

Diet supplement	Iodine value	Molecular weight (Rast)	Growth[2] ratio
Fresh corn oil fatty acids	126.0	294	1.000
Thermally oxidized fatty acids	70.1	459	+0.285
Urea adduct forming fatty acids from T.O.[3] oil	62.0	300	+0.989
Non urea adduct forming fatty acids from T.O. corn oil	83.0	512	−0.020
Molecular distillate of non adducting acids from T.O. corn oil	113.0	320	+0.965
Non distillable residues from non adducting acids from T.O. corn oil	70.0	630	−0.090

[1] Corn oil heated for 48 hrs. at 200° C with 150 ml of aeration/min/kg of oil.

[2] Growth ratio = $\frac{\text{weight gain on test diet in grams}}{\text{weight gain on fresh corn oil diet in grams}}$.

[3] T.O. = thermally oxidized corn oil.

at the 10% level for 15 weeks. A significant growth depressing effect was noted when the growth of rats on the test diets of used corn oil and lard was compared to that of the rats on test diets of corresponding fresh oils. The used hydrogenated vegetable shortening gave the least amount of growth depression. It was also reported that the effects were partially overcome by increased pyridoxine and riboflavin supplementation.

On the other hand, Melnick and co-workers have presented evidence that oils in commercial usage in continuous potato chip fryers are free of thermal polymers because no statistically significant difference was found in the following constants: iodine value, melting point, settling point, fatty acids composition, free fatty acid content, content of isomerized acids, and solids content index. In another publication, the same author, reviewing the literature concerning heated fats, has expressed the opinion that most of the findings are irrelevant to practical operations. A change of 1% in the iodine value was noted in oils obtained from 89 potato chip manufacturers. This change was found not to be statistically significant, and on the basis of this the authors conclude that these used oils are free from thermal polymers *(72, 73, 74)*. However, Melnick did not feed these oils and could therefore not draw any valid conclusions in regard to their nutritive value. Furthermore, he did not isolate the polymers and determine their nutritional characteristics.

Recent reports by Keane *et al (61)* have shown that hydrogenated cottonseed oil obtained from commercial frying operations and fed to rats at the 20% level showed no adverse effects. It was also shown that thermally oxidized oils prepared on a laboratory scale caused growth depression, but no toxic symptoms were noted. A similar report by Rice, Mone and Poling *(92)* also indicated that used oils may not be nutritionally harmful to rats. These authors studied 34 samples of fat from various restaurants, bakeries, potato chip, and doughnut manufacturers to determine the influence of usage on nutritional value. The most severely abused of the oils examined caused a slight decrease in the caloric availability of the fat and a slight increase in liver size. These authors stated that their findings did not support the hypothesis that used fats may be harmful. These results suggested that the commercial fats studied were more resistant to nutritional change and that conditions of commercial usage (heating, aeration) are less drastic than laboratory heated oils.

It is necessary at this point to discuss briefly the chemistry of the various types of polymerizations. The modern concept of autoxidation is presented in Figure 3 *(6, 7, 9–11, 16–20, 23, 24, 32, 36–39, 42–44, 50, 52, 58, 59, 63, 66, 67, 71, 86, 89, 90, 101, 102, 105, 106, 109)*. Autoxidation of ethyl linoleate with oxygen leads to the formation of conjugated ethyl linoleate hydroperoxides which can in turn cyclize and form higher perioxidic polymers or combine in other ways to form non-cyclic branched chain products. Such peroxides may also decompose by addition to a carbon carbon double bond with the formation of other hydroxyl and epoxy compounds. Hydroperoxides may, if formed during the course of autoxidation, recompose to ketones, hydroxy epoxy, and keto hydroxy products. If unsaturated peroxides are present, both saturated and unsaturated aldehydic and ketonic products may arise. An excellent review by Holman, which covers the subject of autoxidation more completely *(50)*, is available.

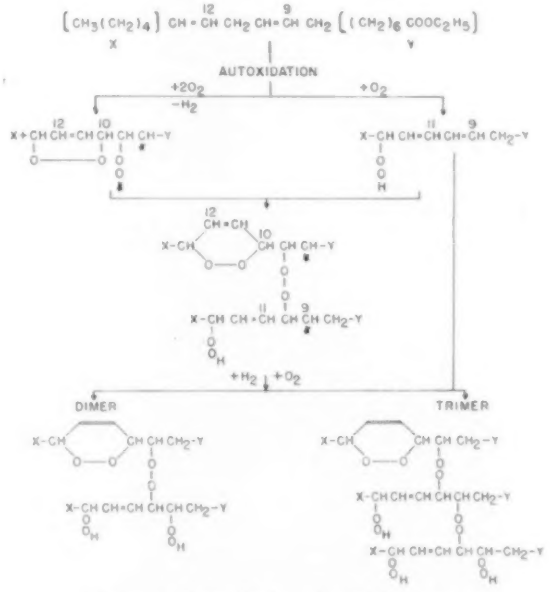

Figure 3. Autoxidation of ethyl linoleate.

The chemistry of thermal polymerization is less complex than that of autoxidation *(8, 12–14, 21, 22, 57, 60,*

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70, 77–80, 103). The changes taking place are primarily those of a normal Diels-Alder reaction (Figure 4). Polyunsaturated acids such as linoleic, linolenic, and eleostearic acids undergo thermal polymerization after conjugation to form polymeric products. One may conclude from the published literature that thermal polymerization of an unsaturated oil results in the formation of cyclic monomers, dimers, trimers, and even higher polymers. Several other compounds are formed in small amounts as a result of the interaction of shorter chain compounds with normal fatty acids *(12)*. The interaction of 1-octene, methyl, 9-decenoate and 1,3-octadiene with methyl oleate and linoleate can produce complex Diels-Alder adducts, when they are formed from degradation products of oleic and linoleic acids.

Thermal oxidation. The chemistry of oils oxidized at high temperatures is more complex than that of either thermal or oxidative polymerization since both heat and oxygen are involved. Taylor has reviewed the chemistry of oxidized oils in industrial products *(104)*. The high temperature oxidation of linseed oil has been reviewed by Hess and O'Hare, who indicated that the oxidation of linseed oil at high temperatures involved free radical mechanisms similar to those proposed for autoxidation *(46)*. These authors stated that hydroxyl groups were initially formed and subsequently esterified by a free carboxyl group of another fatty acid molecule. From this type of reaction they concluded that active hydroxyl groups were formed during the oxidation of linseed oil which could in turn react to form other compounds and eventually result in the final oxidation products. These results have received further confirmation from the work of Ellis *(35)*, who identified hydroxyl groups in dried linseed oil films. Moreno and Lopez *(75)*, who oxidized cottonseed and olive oil at 120° C in the presence of air and manganese dioxide catalyst, produced hydroxylated compounds and subsequent polymerization. Oxidation of brassidiic acid at 130° C yielded alpha dihydroxy behenic acid and ketohydroxy acids *(100)*. Oxidation of paraffin distilaltes has also been shown to produce hydroxylated materials of varying chain length *(111)*.

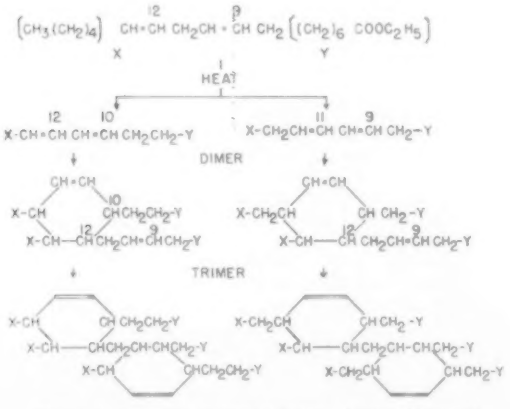

Figure 4. Thermal polymerization of ethyl linoleate.

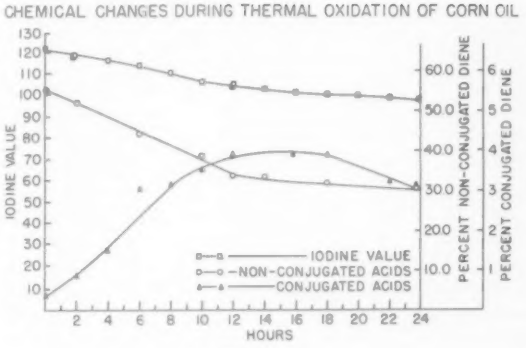

Figure 5.

Johnson and Kummerow have studied the chemical changes that take place during thermal oxidation of edible oils *(51)*. They found that unsaturation decreased, conjugation decreased, and non-conjugated acids increased (Figure 5). Positive evidence for the formation of unsaturated carbonyl compounds was also obtained. This work has been confirmed recently by Dangoumau and Associates in France *(31)*. In general, therefore, the literature suggests that the high temperature oxidation of oils leads to hydroxylated materials of varying molecular weight.

Recently, a more comprehensive study of thermally oxidized oils was reported. The types of compounds formed when corn oil was subjected to temperatures of 200° C with aeration were studied by Perkins and Kummerow *(85)*. Corn oil was oxidized at 200° C for 48 hr with aeration, separated into several fractions, and partially characterized. The fractionation of the oil was carried out by first treating the corresponding acids with urea to remove unoxidized material. The non-urea adduct-forming material was then subjected to molecular distillation. The non-distillable material was also subjected to a crude solvent fractionation to separate the more polar components. As the polymer content increased the Rast Molecular weight also increased while the iodine values decreased (Figure 2). Analyses of the fractions obtained indicated that the oxygen was present in the form of hydroxyl and carboxyl groups. No evidence for the presence of cyclic compounds was obtained, thus decreasing the possibility of Diels-Alder reactions occurring to any extent during thermal oxidation. It appears that the thermal oxidation of corn oil produced various types of polymeric material of high molecular weight and high oxygen content which caused growth depression when fed to rats.

IMPLICATIONS OF THE RESEARCH ON HEATED FATS

Practical research concerning the formation and accumulation of polymeric materials in oils obtained from deep frying operations has not received enough emphasis in published work. Literature concerning chemical determination of polymeric materials in fatty acids obtained from commercial operations is not readily available.

It can also be seen from the foregoing discussion that differences in the temperatures of heating and oxygen content can alter the rate and types of polymerization. The length of time that an oil is exposed to oxygen and heat, and especially the percentage of heated oil in the diet, seems to have as great a bearing on the nutritive value of the oil as temperature of heating.

In summary, it can be observed that most of the data derived from this type of research cannot be projected to predict whether an unsaturated oil will be damaged sufficiently during processing and commercial frying operations to be harmful. The data obtained is sufficient, however, to justify the suspicion that the use of such oils containing polymeric materials may not be desirable from a nutritional point of view. It is obvious that practical research must yet be done in order to answer at least three main questions: A) Are polymers formed in unsaturated oils during deodorization, processing and use? B) Are polymeric materials absorbed on food products and if so to what extent? C) What are the physiological and nutritional effects of these materials?

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Consumer Opinion of Sweeteners in Frozen Concentrated Lemonade and Orange Juice Drink*

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FROM THE CONSUMER'S VIEWPOINT, flavor is the most important characteristic of citrus juices *(13)*. Flavor and resultant palatability of citrus juices depends largely upon its sugar content, free acidity, and peel oil level. In the citrus industry, the ratio of soluble solids to total acidity (Brix/acid) is widely used as an index of palatability and maturity in citrus fruits *(1, 7)*.

No consumer preference data for frozen orange juice was found in the literature although several studies on preferences for canned orange juice varying in Brix-acid ratios have been reported *(2, 4, 5, 8, 9)*. An untrained laboratory panel judged samples varying in Brix-acid ratios ranging from 8.2 to 18.8 at 12.6° Brix *(2)*. The closest ratios that this panel could distinguish were between 12.4 and 8.2, and between 13.7 and 17.5. The sample with the highest ratio, 18.8, was preferred. Using a 10-point scale, members of 695 households in Indianapolis, Indiana gave preference ratings to 6 canned orange juices ranging in Brix-acid ratios from 12 to 22 at 12° Brix *(5)*. Although the results were bimodal and there was an absence of a sharp preference pattern, samples with a ratio of 18 and 20 received the highest scores. Employing a consumer panel of 160 persons, Morse *(8)* found that canned orange juices with ratios of 12 and 22 were disliked; the greatest preference was for samples within the range of ratios of 14 through 20.

Frozen type concentrates for lemonade were introduced in 1949 and have had rapid public acceptance. According to the U. S. standards for grades *(12)* frozen concentrate for lemonade is the product prepared from lemon juice and one or more nutritive sweetening ingredients; it may contain added lemon oil or concentrated lemon oil and may or may not contain water in sufficient quantities to standardize the product. For "A" classification, lemonade must test not less than 10.5° Brix, 0.7% acid and the Brix/acid should not exceed 20:1. Cole *(6)* reported the following range and mean values for sugar and acid levels for 30 brands of lemonade concentrate:

	Concentrate for lemonade		Lemonade	
	Range	Mean	Range	Mean
Brix	48.5–55.7	52.1	10.66–12.56	11.61
Citric acid anhyd. % (W/W)	3.06–3.58	3.31	0.69–0.79	0.7
Brix/acid	14:1–18:1	16:1	14:1–18:1	16:1

Cole *(6)* believes that because Bayton and Bell *(3)* found consumers could not discriminate positively between the sweetest and the most tart concentrated lemonade products on the market, that consumer preference tests should be confined to factors other than Brix and Brix/acid ratio. It should be pointed out, however, that these consumers were 18 untrained laboratory tasters who evaluated the following samples by the duo-trio method: A—10° Brix, 11.1 B/A; B—10° Brix, 16.6 B/A; C—12.6° Brix, 14.0 B/A; D—12.6° Brix, 21.0 B/A. The panel was unable to distinguish between samples A and B and between A and C, but all other paired combinations were readily distinguishable. Sample C was preferred. Morse *(9)* feels that the consumer can indeed detect such essential quality factors as sweetness and acidity (in canned orange juice).

In the present study, three consumer surveys were conducted to determine opinion of amount and type of sweetener in frozen, concentrated lemonade and in frozen, concentrated orange juice drink:

Study I. Determination of optimum sweetness in sucrose-sweetened concentrates.

Study II. Effect on acceptance of dextrose replacement of 10, 20, and 30% of the sucrose.

Study III. Effect on acceptance of corn syrup replacement of 10, 20, and 30% of the sucrose.

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EXPERIMENTAL PROCEDURE

Preparation of the orangeade and lemonade concentrates was carried out in the pilot plant of the Department of Food Science and Technology. The bases which entered into their preparation had the following composition:

	Lemon concentrate[b]	Orange concentrate[c]
Brix	45.69	42.1
Acid, % anhyd. citric (w/w)	27.41	3.8
Recoverable oil, % (v/v)	0.293	0.020
Pulp,[d] % (v/v)	14	None

[b] Furnished by Research Dept., Sunkist Growers.
[c] Furnished by Golden Citrus Juices, Inc.
[d] Retained on a 20 mesh screen.

The bases were shipped frozen and were held in the frozen condition until used for the preparation of the test samples.

All-sucrose pack for Study I. Lemonade concentrates were prepared to give 4 levels of sweetness following a 1 plus 4 dilution with water; namely 9, 10, 11, and 12° Brix, respectively. The frozen lemon concentrate was thawed and thoroughly mixed before transferring measured amounts by weight to the stainless steel blending tank. The granulated sucrose and chilled (40° F) tap water entering the formulations were also weighed and thoroughly mixed with the concentrate. Each lot was filled, by exact weight, into 5 oz enameled cans, sealed under steam-jet closure and immediately frozen. Acid served as a base for calculating the formulations for the lemonade concentrate. Each of the 4 sucrose levels were formulated to contain 0.7% acid as anhydrous citric in the dilute drink.

The orangeade concentrates were prepared in a similar manner but with the modification noted below. The 4 levels of sweetness chosen for testing were 11, 12, 13, and 14° Brix in the final drink following a 1 plus 3 dilution of the orangeade concentrate with water. In addition to the sugar and water, citric acid (U.S.P.) and orange oil entered into the formulation. The orange oil (18 ml/10 gal concentrate) was blended with the dry sucrose, then thoroughly dispersed throughout the entire blend of ingredients.

Sucrose-replacement packs for Studies II and III. Using the results of Study I as a basis, lemonade and orangeade concentrates were prepared with 10, 20, and 30% of the sucrose replaced with dextrose [e] for Study I and with corn syrup [f] for Study II.

The 4 lemonade and orange samples for each study were prepared in the same manner used for the all-sucrose packs. Each formulation was prepared separately and carefully to exact weight.

Consumer survey. Three separate surveys were conducted among families living in Woodland, an agricultural city of 4,000 families situated 70 miles northeast of San Francisco. For Study I, conducted in May, 1959, 400 families randomly selected from the telephone directory were invited to participate. Subsequent surveys, Studies II and III conducted in June and July, employed those families from the original sampling who were willing to be retested. The number of families participating and the per cent returning their completed score cards are as follows:

	Study I	Study II	Study III
Total No. families participating...	320	300	269
Percent return...	95.9	98.0	98.1

At each of the 3 testing periods, every family received 2 cans of frozen lemonade concentrate and 2 cans of frozen orangeade concentrate. For the all-sucrose pack, the six possible paired comparisons were randomly distributed to the participating families. In the samples containing dextrose or corn syrup, no cross comparisons were made; the all-sucrose controls were compared against their respective replacement series, only.

The identification on the cans consisted of the words "Orange" or "Lemon" and codes "Green" or "Red" to distinguish samples within a pair. The homemaker was instructed to place the concentrates in the freezer part of the refrigerator and to evaluate them within 2 days after delivery. To insure correct reconstitution, the following directions were given "Lemonade—Place contents of each can in separate containers. To each add 4 level cans of cold water. Stir, chill, stir again, then taste. Orangeade—Place contents of each can in separate containers. To each add 3 level cans of cold water. Stir, chill, stir again, then taste. It is extremely important to measure water correctly." Each family member over the age of 6 received an orange-striped score card and a yellow-striped score card which read as follows:

Family Number.....

SEX:
Male.....
Female.....

AGE:
6-12.....
13-20.....
21-40.....
Over 40.....

State reason for preference on reverse side.

PREFERENCE (check only one)

I prefer GREEN over red:
Extremely.....
Very much.....
Moderately.....
Slightly.....

I prefer RED over green:
Extremely.....
Very much.....
Moderately.....
Slightly.....

I have NO PREFERENCE.....

The samples within each pair were coded in reverse order in half of the cases to offset any code bias or first-sample bias. Stamped, addressed envelopes were provided for mailing the completed score cards. Preferences were analyzed by Chi Square, using Yates' correction for continuity *(14)*. Analysis of variance for paired comparison as described by Scheffe *(10)* was applied and will be reported elsewhere.

Laboratory taste panel. A preliminary laboratory taste panel of 18 untrained judges was used to establish degree of difference in the citrus concentrates. The first 2 taste sessions were utilized to acquaint the judges with the commodity and the triangle test methodology. At subsequent sessions, evaluations were made of the samples in the same combination as presented to the consumer panel. Judging was done at mid-afternoon in individual booths under red illumination which effectively masked differences in appearance. A second laboratory taste panel, consisting of the 5 most sensitive tasters from the previous panel, judged the relative sweetness in the lemonade concentrates using a paired comparison methodology.

RESULTS AND DISCUSSION

Composition of the concentrated and reconstituted lemonade and orangeade is shown in Tables 1 and 2. Brix values for the reconstituted products are slightly under the intended levels but differ by 1° Brix for the series in Study I.

Consumer panel. Distribution of the participating consumers by age and sex (Table 3) showed that approximately 30 more females than males were interviewed. There were more people represented in the age group of over 40 than in the groups including young adults, teenagers, or children.

Preferences for samples varying in sucrose content are shown in Table 4. Sample "C" for both lemonade and orangeade, with Brix levels of 10.72° and 12.93°, respectively, received the greatest number of comparative preferences. The samples placing second in degree of liking had Brix levels of 9.82° and 13.93° for

[e] Baker and Adamson, reagent grade anhydrous dextrose.

[f] American Maize-Products Co.; analysis: acid converted, 52.5 D.E., 43.0 Be, 80.7% total solids. Protein 0.03, Ash 0.24, Iron (as Fe) 1 ppm, Lead (as Pb) −1 ppm, Acidity 0.009, SO_2 (M.W.) 0.0008, pH (1:1) 4.1, O.D. 0.039, Heat test 0.114. Bacteriology analysis: Mold/g 0, Yeast/g 13, Bacteria/g 33, Flat sours/10g 20, H_2S formers/10 g 0. This syrup meets NCA specifications.

TABLE 1

Composition of concentrated and reconstituted lemonade one week after processing

	Study I All sucrose pack				Study II Dextrose replacement				Study III Corn syrup replacement			
	A	B	C	D	0 E	10% F	20% G	30% H	0 I	10% J	20% K	30% L
Concentrate												
° Brix	39.36	43.11	46.51	50.51	46.58	46.68	46.98	46.98	45.98	46.58	46.58	46.58
Acidity[1]	3.10	3.06	2.87	3.01	3.00	2.93	3.00	3.06	3.01	3.03	2.89	2.94
pH	2.3	2.4	2.3	2.3	2.6	2.6	2.5	2.5	2.6	2.6	2.6	2.6
Brix-acid ratio	12.69	14.08	16.19	16.79	15.55	15.94	15.65	15.34	15.28	15.38	16.12	15.83
Reconstituted[2]												
° Brix	8.71	9.82	10.72	11.83	10.70	10.85	10.85	10.85	10.70	10.85	10.75	10.75
Acidity[1]	0.69	0.68	0.67	0.70	0.70	0.69	0.70	0.71	0.70	0.71	0.68	0.69
pH	2.5	2.5	2.5	2.5	2.8	2.7	2.8	2.8	2.7	2.8	2.8	2.8
Brix-acid ratio	12.62	14.38	16.00	16.83	15.31	15.82	15.50	15.24	15.24	15.37	15.86	15.63

[1] Total acidity calculated as per cent citric acid anhydride.
[2] 120 ml concentrate + 480 ml tap water.

TABLE 2

Composition of concentrated and reconstituted orange juice drink one week after processing

Code	Study I All sucrose pack				Study II Dextrose replacement				Study III Corn syrup replacement			
	A	B	C	D	0 E	10% F	20% G	30% H	0 I	10% J	20% K	30% L
Concentrate												
° Brix	38.94	42.94	45.44	47.94	45.78	45.48	45.08	45.58	45.48	45.98	46.48	45.48
Acidity[1]	2.47	2.49	2.42	2.40	2.43	2.45	2.43	2.43	2.44	2.44	2.42	2.44
pH	2.9	2.9	2.9	2.9	3.00	3.0	3.0	3.0	3.00	3.1	3.00	3.0
Brix-acid ratio	15.78	17.22	18.74	19.98	18.88	18.56	18.57	18.77	18.64	18.88	19.17	18.65
Reconstituted[2]												
° Brix	10.92	12.03	12.93	13.93	12.90	12.90	12.80	12.75	12.75	12.80	12.90	12.65
Acidity[1]	0.70	0.71	0.70	0.70	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
pH	3.0	3.0	3.0	3.0	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1
Brix-acid ratio	15.67	16.97	18.55	20.04	18.27	18.09	18.10	18.01	17.96	18.05	18.27	17.82

[1] Total acidity calculated as per cent citric acid anhydride.
[2] 120 ml concentrate + 360 ml tap water.

TABLE 3

Age and sex distribution of participating consumers

Age	Study I			Study II			Study III		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
Lemonade									
6–12	87	95	182	92	94	186	84	99	183
13–20	66	57	123	66	61	127	60	54	114
21–40	124	151	275	118	140	258	106	130	236
Over 40	182	188	370	164	168	332	142	155	297
Total	459	491	950	440	463	903	392	438	830
Orange drink									
6–12	94	94	188	94	90	184	82	99	181
13–20	66	60	126	62	64	126	59	53	112
21–40	122	147	269	116	139	255	108	126	234
Over 40	185	192	377	160	172	332	149	151	300
Total	467	493	960	432	465	897	398	429	827

TABLE 4

Consumer preference for frozen citrus concentrates at various sucrose levels
(Values represent individual preferences)

Code	Lemonade (950 evaluations)					Orangeade (960 evaluations)				
	A	B	C	D		A	B	C	D	
° Brix	8.71	9.82	10.72	11.83	N.P.[1]	10.92	12.03	12.93	13.93	N.P.[1]
Pair										
AB	46	114†			9	72	80			16
AC	60		72		17	61		77		26
AD	74			77	9	48			85†	16
BC		55	91**		21		54	83*		19
BD		62		66	16		81		66	27
CD			67	69	25			66	60	23

[1] N.P. = No preference.
* Significant preference at the 5% level of probability.
** Significant preference at the 1% level of probability.
† Significant preference at the 0.1% level of probability.

TABLE 5

Consumer preference for frozen citrus concentrates with various replacements of sucrose by dextrose (Values represent individual preferences)

	Lemonade (903 evaluations)					Orangeade (897 evaluations)				
Code	E	F	G	H		E	F	G	H	
% Dextrose replacement	0	10	20	30		0	10	20	30	
Pair					N.P.[1]					N.P.[1]
EF	131	115			40	107	128			67
EG	121		126		58	124		102		66
EH	160†			105	47	135			125	43

[1] N.P. = No preference.
† Significant preference at the 0.1% level of probability.

TABLE 6

Consumer preference for frozen citrus concentrates with various replacements of sucrose by corn syrup (Values represent individual preferences)

	Lemonade (830 evaluations)					Orangeade (827 evaluations)				
Code	I	J	K	L		I	J	K	L	
% Corn syrup replacement	0	10	20	30	N.P.[1]	0	10	20	30	N.P.[1]
Pair										
IJ	126*	90			64	140†	81			42
IK	139†		86		54	123		97		55
IL	154†			96	21	127			105	57

[1] N.P. = No preference.
* Significant preference at the 5% level of probability.
** Significant preference at the 1% level of probability.
† Significant preference at the 0.1% level of probability.

lemonade and orangeade, respectively. It is not possible to determine from the data whether the insignificant preference for pair CD is due to inability to distinguish sweetness or to disagreement in sweetness preferences among the consumer group tested.

The effect of dextrose and of corn syrup replacement on consumer opinion differed for the two products. For lemonade, only replacement of 30% of the sucrose by dextrose produced a significant preference for the all-sucrose control (Table 5). No significant preferences were obtained for orangeade containing dextrose when compared with the control. It should be pointed out, however, that 6 months after storage at 0° F., an undesirable darkening, which increased with increasing dextrose replacement, was observed in both lemonade and orangeade samples F, G, and H. The change in color was not so evident in the concentrates sweetened with sucrose, corn syrup, or a combination of sucrose and corn syrup. A study of the effect of various grades and levels of dextrose substitution on the color of the concentrates is being planned.

Data in Table 6 indicate a significant rejection of the lemonade concentrate containing corn syrup. Preferences for orangeade within this series presents a confusing picture since the all-sucrose control is significantly preferred over 10% corn syrup replacement but not over 20 or 30% replacement. Further breakdown of the preferences (Table 7) shows a similar pattern between sexes and among the age groups, i.e., significant dislike for sample J but not for samples K and L. This phenomenon should be investigated further before definite conclusions can be drawn regarding the effect of corn syrup replacement on consumer acceptability of orangeade. Simon *(11)* reported on a study of orange juice concentrate in which corn syrups of 42 D.E. and 67 D.E. were substituted for sucrose at 25%, 33%, and 50% replacement levels. This investigator concluded that the corn syrups could be substituted for 50% of the sucrose without impairment of flavor or color.

TABLE 7

Distribution of preferences for orangeade Study III

Code	I	J	K	L	
% Corn syrup	0	10	20	30	N.P.[1]
Males					
IJ	63*	41			21
IK	61		53		21
IL	63			49	26
Females					
IJ	77†	40			21
IK	62		44		34
IL	64			56	31
Age 6–12					
IJ	34	21			8
IK	28		19		7
IL	25			28	11
Age 13–20					
IJ	13	5			4
IK	13		16		11
IL	19			23	8
Age 21–40					
IJ	46**	20			15
IK	33		27		13
IL	39			23	18
Over 40					
IJ	47	35			15
IK	49		35		24
IL	44			31	20

[1] No preference.
* Sig. at P = 0.05.
** Sig. at P = 0.01.
† Sig. at P = 0.001.

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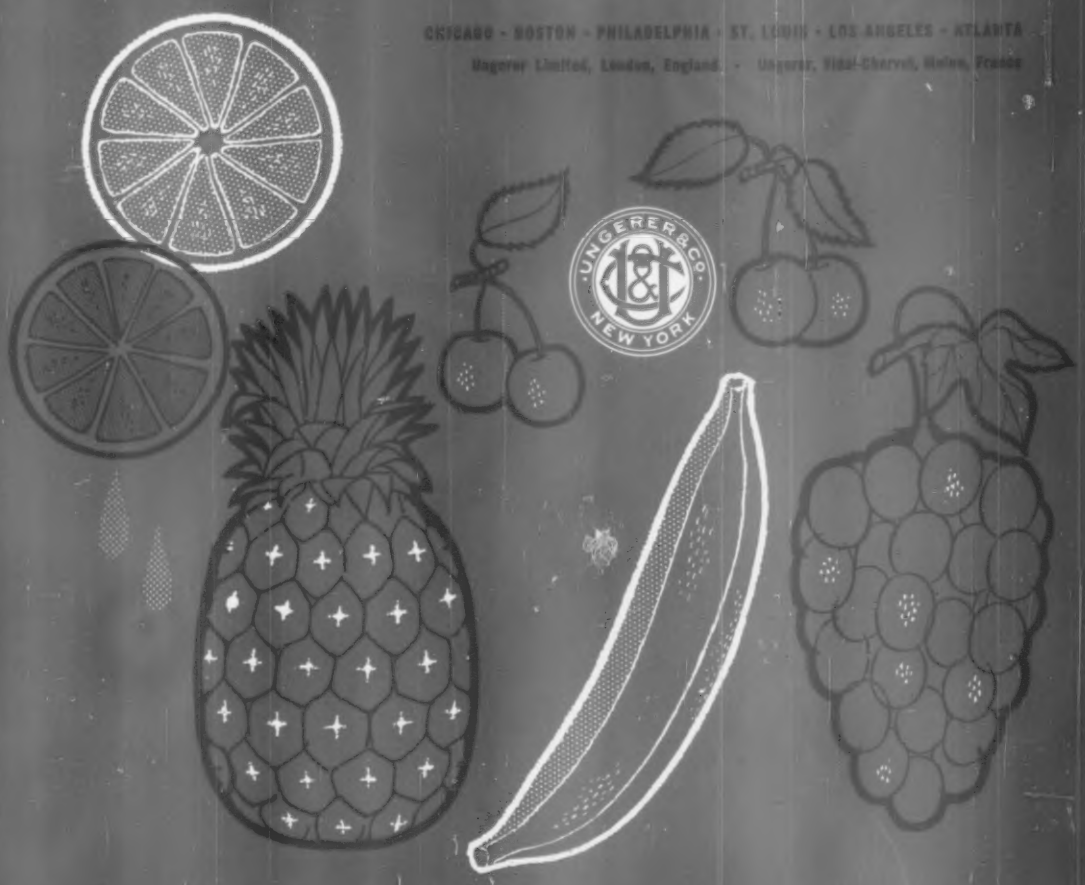

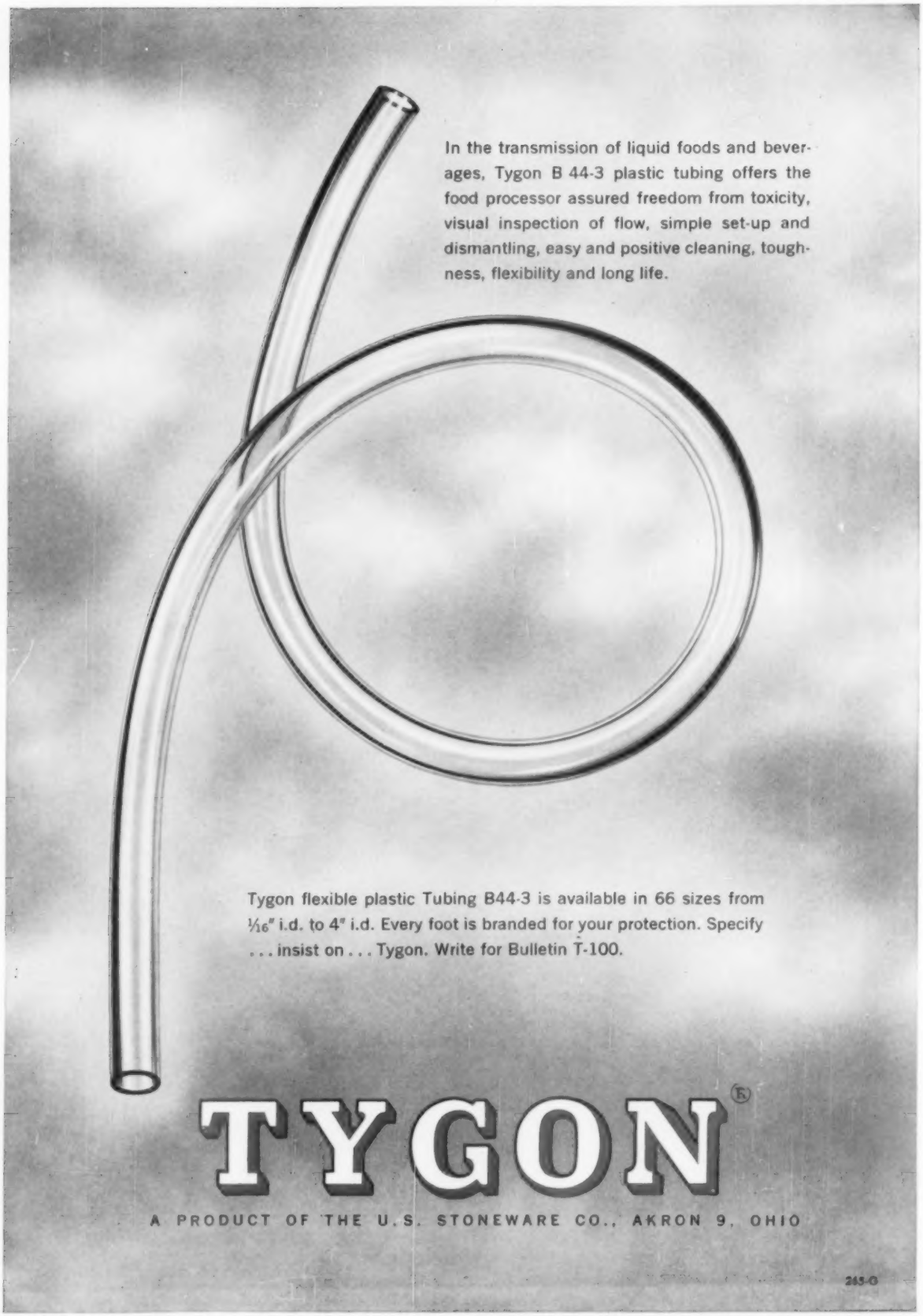
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TABLE 8

Consumer comments (frequency of occurrence)

Comment	Code				Code				Code			
	A	B	C	D	E	F	G	H	I	J	K	L
	Sweetener[1]				Sweetener[1]				Sweetener[1]			
	S	S	S	S	S	10%D	20%D	30%D	S	10%C	20%C	30%C
Lemonade												
Better flavor	31	43	47	32	49	18	24	17	58	8	10	8
Poor flavor	27	6	13	10	11	8	10	4	9	15	8	7
Sweeter	6	47	56	61	81	12	15	9	63	9	4	6
Too sweet	5	3	13	37	39	10	3	4	23	7	5	3
Too sour	21	16	2	5	11	6	3	4	13	5	10	8
More body	2	5	3	4	3	1	3	0	2	1	3	0
Orangeade												
Better flavor	63	42	43	51	70	29	14	24	67	18	11	15
Poor flavor	27	17	4	11	14	9	8	9	21	7	9	11
Sweeter	20	26	32	50	47	8	1	3	64	15	10	16
Too sweet	15	14	34	44	41	9	8	10	23	9	8	3
Too sour	14	13	4	2	7	2	4	5	10	7	2	4
More body	4	2	6	7	5	0	0	2	5	1	2	1

[1] S = sucrose, D = dextrose replacement, C = corn syrup replacement.

TABLE 9

Laboratory panel discrimination
(Triangle method)

Comparison	Lemonade		Orangeade	
	Number judgments	Correct separation	Number judgments	Correct separation
A B	16	10*	16	14†
A C	16	13†	16	15†
A D	15	14†	17	16†
B C	15	9*	17	10*
B D	15	15†	14	11†
C D	15	11**	14	6
E F	17	6	17	11**
E G	14	7	16	11**
E H	15	7	14	9*
I J	15	5	11	5
I K	16	8	16	7
I L	17	10*	16	12†

* Sig. at P = 0.05.
** Sig. at P = 0.01.
† Sig. at P = 0.001.

Flavor was the quality factor most frequently given as a reason for selection of one sample over another (Table 8). A similar pattern of response was noted for both citrus products. A relatively large number of people found sample A to have poor flavor. Sweetness was frequently mentioned and undoubtedly influenced the response obtained in Studies II and III. Throughout all 3 studies, only 7 comments regarding color were received, indicating the darkening observed after 6 months of storage was not apparent one week after packaging.

Laboratory panel. Using the triangular method, the laboratory panel was able to distinguish between all samples within Study 1 except for orangeade samples C vs D (Table 9). In most cases, the all-sucrose, orangeade control was found to be significantly sweeter than the samples containing dextrose or corn

TABLE 10

Relative sweetness of lemonade[1] (values represent percentage of response to sweetness)

Code	A	B	E	F	G	H	J	K	L
°Brix	8.7	9.8	10.7	10.7	10.9	10.9	10.9	10.8	10.8
Sweetener[2]	S	S	S	10%D	20%D	30%D	10%C	20%C	30%C
A B	37.5	62.5							
A E	18.7		81.3**						
A F	0.0			100†					
A G	0.0				100†				
A H	14.3					85.7†			
A J	0.0						100†		
A K	26.7							73.3*	
A L	26.7								75.0**
B E		6.7	93.3†						
B F		7.7		92.3†					
B G		58.5			41.5				
B H		62.5				37.5			
B J		31.2					68.8*		
B K		46.4						53.6	
B L		90.0†							10.0
E F			61.4*	38.6					
E G			78.6**		21.4				
E H			80.0**			20.0			
E J			60.0				40.0		
E K			93.8†					6.2	
E L			88.2**						11.8
F G				62.5	37.5				
F H				65.0		35.0			
F J				50.0			50.0		
G H					57.5	42.5			
G K					70.0*			30.0	
H L						85.0†			15.0
J K							60.0	40.0	
J L							87.5†		12.5
K L								92.5	7.5

[1] Average of 35 to 57 replications. [2] S = all sucrose, D = dextrose replacement, C = corn syrup replacement.
* Sig. at P = 0.05.
** Sig. at P = 0.01.
† Sig. at P = 0.001.

syrup. Since the triangle methodology did not show significant differences between lemonade samples in Studies II and III, the more sensitive method of paired comparison was used for presenting samples to 5 judges selected for their high acuity for sweetness discrimination. Results obtained are presented in Table 10. A difference in soluble solids of 1° Brix within the all-sucrose pack was readily detected. Sample B at 9.8° Brix was equivalent in sweetness to samples G and K at 10.8° Brix with sucrose replacement of 20% dextrose and 20% corn syrup, respectively. Replacement of sucrose with dextrose or with corn syrup definitely reduced sweetness at the same solids level. Comparison of values for pairs FJ, GK, and HL strongly indicates that at the same solids level, dextrose and corn syrup may have the same sweetness at low concentrations but dextrose is sweeter than corn syrup at higher concentrations.

SUMMARY

Three surveys were conducted among 320 families to determine consumer opinion of amount and type of sweetener in frozen, concentrated lemonade and in frozen, concentrated orangeade: Study I—Determination of optimum sweetness among 4 sucrose-sweetened concentrates, Study II—Effect on acceptance of dextrose replacement of 10, 20, and 30% of the sucrose, Study III—Effect on acceptance of corn syrup replacement of 10, 20, and 30% of the sucrose.

In Study I, an optimum sweetness level of 11° Brix at a Brix-acid ratio of 16.0 was established for lemonade whereas 13° Brix at a Brix-acid ratio of 18.5 was optimum for the orangeade. Only the lemonade sample containing 30% dextrose replacement was rejected by the consumers; all orangeade containing dextrose was acceptable. However, undesirable darkening was observed in all dextrose-containing samples after 6 months of storage at 0° F. Corn syrup was objectionable in lemonade, but inconclusive results were obtained for orangeade.

The laboratory taste panel found that 9.8° Brix in the all-sucrose sweetened lemonade was equivalent in sweetness to 10.8° Brix in samples with sucrose replacements of 20% dextrose or 20% corn syrup. Sucrose had the greatest sweetening capacity and corn syrup had the least.

Acknowledgments

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Experimental Preservation of Poultry by Chlortetracycline and Radiation

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(Manuscript received January 19, 1960)

CHLORTETRACYCLINE and oxytetracycline are now commercially used to retard microbial spoilage of fresh poultry. Methods of applying antibiotics to poultry preservation as well as microbiological aspects of the process have been described *(2)*. In general, the shelf-life of refrigerated poultry has been substantially improved with the aid of antibiotics. Experimental work has also demonstrated that ionizing radiations can extend the storage life of poultry by retarding microbial spoilage. Use of 100 to 500 kilorads of radiation has been evaluated as a means of pasteurizing whole eviscerated chickens *(3)*. Combined use of radiation and antibiotics for preserving fresh meats has been reported *(1, 4)* as a means of further improving their shelf-life. Incorporating radiation into an antibiotic preservation process should result in the inhibition of many microorganisms resistant to antibiotics.

Results given here were derived from the experimental preservation of fresh chicken by means of chlortetracycline and X-irradiation. Effects of this process on microbial counts of chicken during refrigerated storage are presented.

MATERIALS AND METHODS

Samples of chicken breast meat were prepared as follows. Sixteen 40-g samples were cut from 8 chickens and placed in polyethylene bags. Samples were approximately 6 cm in diameter and 1.5 cm in thickness. Chlortetracycline (CTC) in 1 ml of water was pipetted into the package which was then heat-sealed and the bag rotated so as to impregnate all meat surfaces with the antibiotic solution. The final concentration of CTC in the meat was calculated to be 10 ppm. Three 40-g samples were used for each parameter of the experiment. Specified samples were irradiated with 100 kilorads of 250 kv X-rays at 1.15 ma from a model OX-250 X-ray machine (General Electric Company); no added filtration was employed. A Plexiglas plate having a center well for the sample supported the latter during irradiation. This plate was attached directly to the X-ray machine. Radiation dosage was determined with 40 ml of a ferrous sulfate solution *(5)* in a polyethylene package having the geometry and size of the packaged meat samples. The latter were stored at 41° F (5° C) for 7 weeks and the total viable counts of microorganisms determined. Initial microbial counts prior to treatment and storage were also obtained. Microbial counts were performed on 5-g portions of meat comminuted in 95 ml of sterile water for 5 min in a Waring blender and 1 ml aliquots were plated in triplicate on trytone glucose extract agar (Difco). Colonies were counted after incubation of plates 48 hr at 25° C. The entire experiment was repeated on three separate occasions.

After the indicated storage period, all samples were also examined for color, odor and texture.

RESULTS AND DISCUSSION

Table 1 presents the microbiological data on fresh chicken stored under refrigeration for 7 weeks after various specified treatments. Although the independent application of either chlortetracycline or X-rays to the fresh meat significantly retarded the growth of microorganisms, only the combined use of antibiotic and radiation resulted in a microbiologically acceptable product under the conditions employed. Stored meat treated with the combination process was similar to fresh chicken in color, odor, and texture. By contrast, the irradiated meat and that treated only with CTC possessed pronounced yeast odor and some slime.

Although X-rays were utilized in this study, it is expected that similar results would be achieved with gamma radiation or electron beams. Since microbiological spoilage of meat predominantly occurs at exposed surfaces, electron beams of 1 to 2 mev should be adequate when employed in conjunction with antibiotic.

These results are in general agreement with those of Cain *et al (1)* for other fresh meats.

TABLE 1

Microbial counts in fresh poultry breast meat treated with 10 ppm of chlortetracycline and 100 kilorads of x-rays then stored 7 weeks at 41° F

Replicate number	Microorganisms x 10^6/g meat*			
	Initial count	Treatment		
		Chlortetracycline (A)	X-rays (B)	Combined (AB)
Experiment 1				
1	7	180	20	4
2	6	50	15	4
3	10	100	30	2
4	7	65	10	6
5	10	40	10	10
6	4	130	10	15
7	5	80	10	7
Mean	7 ± 2.3	90 ± 49	15 ± 7.7	8 ± 3.8
Experiment 2				
1	5	200	80	7
2	12	210	10	2
3	6	110	60	5
4	7	90	80	15
5	1	220	50	4
6	2	120	60	4
7	2	150	190	5
Mean	5 ± 3.8	160 ± 50	76 ± 55	6 ± 4
Experiment 3				
1	7	110	100	10
2	5	70	100	20
3	4	110	110	30
4	4	90	.....	75
5	5	180	140	20
6	1	130	90	51
7	1	90	130	10
Mean	4 ± 2.2	110 ± 36	110 ± 20	31 ± 24

* Values are means of triplicate plate counts. All untreated controls were spoiled after 7 weeks (ca 10^9 microorganisms/g). Mean values are ± standard deviations.

SUMMARY

Ten ppm of chlortetracycline and 100,000 rads of ionizing radiation were used in an experimental antibiotic-radiation process for retarding microbial spoilage in prepackaged fresh chicken meat. Treated meat was stored at 41° F for 7 weeks without microbial spoilage. Results obtained on meat independently treated with either radiation or antibiotic were inferior to those achieved with the combination process.

Acknowledgment

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Time-Temperature Tolerance of Frozen Foods. XXIII.

Quality Changes in Frozen Spinach[a]

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(Manuscript received June 6, 1960)

Types of vegetables have been selected for the investigation of time-temperature effects on retention of quality in frozen foods. Green beans, a pod type, were reported first *(11)*, then peas, a seed type *(5)*, and now spinach, a leafy vegetable.

Some of the effects of constant and fluctuating temperatures near and below the freezing point of water have been determined with regard to rate and extent of changes in chlorophyll, ascorbic acid, physical properties, and organoleptic quality of commercially processed spinach.

MATERIALS AND METHODS

Selected raw material was processed by commercial plants and procedures. Shipping, holding at −20° F, and analysis for reduced ascorbic acid and enzyme inactivation tests were carried out as described for green beans *(11)*. Four small lots of Viroflay spinach grown in California, 2 leaf and 2 chopped, were tested first to determine approximate rates of deterioration. Then two larger lots of 10 oz wax-overwrapped cartons of leaf spinach (Viroflay variety from California and Savoy variety from the Eastern Seaboard) were used to define changes in chlorophyll, reduced ascorbic acid, flavor, reflectance color, and visual color. Four replications or more were used to establish analytical values for chlorophyll and ascorbic acid, and two for reflectance color for each time-temperature exposure.

Chlorophyll. Procedure for green beans *(10)* was modified for most of the samples to shorten extraction time and to avoid cloudiness. The slurry was diluted with 100% acetone just before filtration to give 500 ml of filtrate. Clear filtrate (10 or 15 ml) was diluted with 90% acetone to (100 ml) a desirable optical density for spectrophotometric readings in 5-cm cells. The blank used was 90% acetone. Calculations of the ratio pheophytin::chlorophyll + pheophytin, expressed as percentage, were based on values of $R_o = 0.900$ and $R_{100} = 2.33$ for lots 1, 2, 3, 4, 5 and $R_{150} = 2.25$ for lot 6.

Total carotenes. These were determined in 5 ml of the filtered acetone extract for chlorophyll by transferring to petroleum ether and chromatographing by the procedure of Bickoff *et al (3)*. The carotenes were eluted and diluted to 100 ml. Absorbance was read in 5-cm cells at 451 mμ with a spectrophotometer. A specific absorption coefficient of 251 as determined by Bickoff *et al (4)* was used to calculate mg β-carotene per 100 g frozen spinach.

Subjective appraisal. Packages were thawed 30 min. Contents were cut into 60 cubes and added to 20% by weight of boiling distilled water, heated, and stirred every 15 sec for 2 min and then boiled 1 min without stirring.

[a] Presented at the Nineteenth Annual Meeting of the Institute of Food Technologists, Philadelphia, Pennsylvania, May, 1959.

[b] A Laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

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Three pairs, control and stored sample, preceded by a labeled control, were appraised each session under low-intensity green light. Order of samples and pairs was randomized each day. Four replications of each pair and four or more periods for each temperature condition were appraised by 10 to 14 selected *(7)* judges. Rank 1 indicated no or less off-flavor and rank 2 indicated more off-flavor. Number of days before 75% of judgments concurred that a sample had more off-flavor than the control was obtained from regression of percentage concurrence on days by Guadagni's procedure *(12)*.

Reflectance color. Measurements were made with a Hunter Color and Color Difference Meter (HCM) on samples "as received" by the technique of Boggs *et al (6)* for loading the cells. Although measurement of color on stem only was more uniform, the proportion of stems to leaf was too small for adequate sampling.

Industry evaluations. Quality scores were assigned by the procedure used for green beans *(11)*.

RESULTS AND DISCUSSION

Data characterizing the 6 lots of control material held at −20° F are given in Table 1. Although analytical values vary somewhat in these few lots, there were not sufficient data to reveal any effect due to harvest year, variety, growing area, or processing condition.

Chlorophyll. The relationship between time and percentage of chlorophyll converted to pheophytin in samples held at steady temperatures in the range −10° to 25° F, or at temperatures fluctuating regularly with a 20° amplitude in the range −10° to 20° F, was found to be linear within the precision of the data. Linear regressions as computed for the data of lot 5 are shown in Figure 1. Previous investigations of green beans *(11)* and peas *(5)* had indicated a logarithmic relationship between percentage chlorophyll retention (100 − % conversion), and time. The magnitude of the standard error of estimate (SE) at each temperature is indicated in Figure 1 by the short vertical bar crossing the regression line. Chlorophyll conversion did not increase in samples held at −20° F and the standard deviation (SD) was 1.25% for a total of 29 packages analyzed on 16 different dates throughout a 2-year period.

Rate of chlorophyll conversion increased exponentially with temperature; percentage chlorophyll conversion per day is plotted on a log scale against temperature in Figure 2. Between 25° and 30° F where a large proportion of the material thaws, the increase in rate was interrupted (lot 5) and then the rate increased rapidly again with temperature. A ten-fold

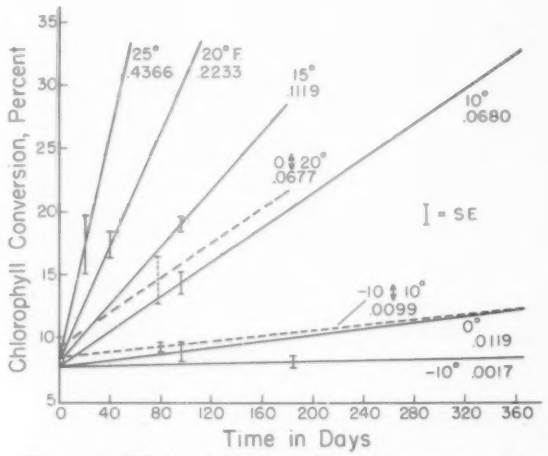

Figure 1. Effects of time and temperature on conversion of chlorophyll to pheophytin.

SE = standard error of estimate for line points. The zero point represents an average of 29 controls. The broken lines represent 24-hour cyclic sine wave fluctuations of air temperatures. The lower solid line (−10° F) represents cased packages; all others were exposed singly. Slopes of the temperature lines are indicated by the coefficients (0.4366, 0.2233, etc.).

increase in rate occurred between horizontal lines—that is, with about 15° increase in temperature.

Fluctuating temperature deterioration rates for chlorophyll, ascorbic acid, and flavor did not differ by more than the precision of estimate from the rate at the effective mean temperature. This agrees with the more detailed data and discussion presented for green beans *(11)*.

Based on only a few data, the chopped spinach, lots 2 and 4, converted chlorophyll to pheophytin at any given temperature at about twice the rate found for leaf spinach, but relative change of rate with temperature was parallel with the change in rate for leaf spinach. Since the processing conditions were the same for both leaf and chopped spinach, packed from the same lot of raw material (lots 1 and 2), it can only be surmised that greater physical manipulation of the chopped spinach created conditions whereby these reactions could take place at faster rates. Careful selection of raw material and a uniform leaf-to-leaf blanching procedure will be necessary for significant correlation of processing conditions with quality retention.

TABLE 1

Identification of lots, processing conditions, and analytical values for −20° F control samples

Lot No.[1]	USDA grade	Harvest year	Variety	Growing area[2]	Blanch conditions[3]		Chlorophyll conversion per cent	Chlorophyll optical density, E[4]	Carotene mg/100 g	Ascorbic acid mg/100 g
					Temp °F	Time min				
1L	A	1955	Viroflay	CC	210–212	3.5	6.9	.24	4.6	44.0
2C	A	1955	Viroflay	CC	210–212	3.5	7.6	.25	4.4	50.5
3L	B[5]	1955	Viroflay	CC	210–212	3.0	8.6	.25	4.3	42.3
4C	A	1955	Viroflay	CC	210–212	3.0	12.8	.20	3.5	38.5
5L	A	1956	Viroflay	CC	210–212	1.5–3	8.4	.23	4.7	47.0
6L	A	1956	Savoy	ES	212	2.5	6.6	.29	5.7	40.2

[1] L = leaf; C = chopped; 1L and 2C packed from the same raw material; 4C packed at the same plant 15 days after 3L.
[2] CC = Central California; ES = Eastern Seaboard.
[3] All lots were adequately blanched in steam and water cooled.
[4] Relative chlorophyll content expressed as optical density reading, E, in a 5 cm cell at 556 mmc for an extract of 1 gram spinach diluted to 100 ml in 90% acetone. Hunter Color and Color-Difference Meter values, HCM, are given in Table 5.
[5] B grade assigned because of number of yellow leaves caused by mildew.

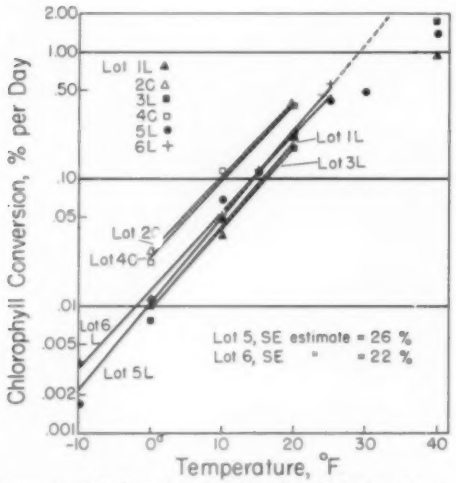

Figure 2. Relationship of chlorophyll deterioration to temperature. Regression lines of rates of chlorophyll conversion (% conv per day) on temperature. Packages were exposed singly, except at −10° F, where they were cased.

Ascorbic acid. When logarithms of mg of ascorbic acid for 100 g of spinach were plotted against time at a given temperature (Figure 3), fairly straight lines were obtained. Rate of loss for the first 10 to 15 mg was often faster than this logarithmic relationship when samples were tested in the −10° F to 20° F range of conditions. A 50 to 75% loss in ascorbic acid occurred in 1 year at 10°; similar losses were noted by Bennett *et al (2)*. The temperature coefficient of rate of loss in the range 0° to 25° F was about the same as for chlorophyll conversion. A regression line for lot 5 data showed a ten-fold increase in rate for a 16° rise in temperature; thus 1 week at 25° would cause as much loss of ascorbic acid as 2 months at 10° F.

Variability among packages was extreme, as shown by the SE of estimate for regression lines for 8 temperature conditions (Figure 3); therefore, only broad trends are comparable. Burger *et al (8)* also showed a broad range in ascorbic acid content among sets of about 35 packages of spinach with an average age of 6.8 months at 0° F. Amount of ice in the packages, ratio of leaf to stem, and difference in concentration of ascorbic acid in these parts are known factors contributing to package variation. A standard deviation of 9 to 11%, with respect to initial amount, per half package based on 20 to 30 controls was more than twice that found for a relatively uniform commodity like peas. This variability made it difficult to determine deterioration rates with good precision.

Moisture loss. A wide variation in moisture loss was found between lots 5 and 6 where 20% and 6% respective losses were noted in material held 1 year at 10° F. Additional observations on weight loss were made on spinach and other vegetables and reported by Nutting *et al (14)*. A general discussion of cause of "freezer burn" and moisture loss is given by Desrosier *(9)*.

Carotenes. Measurements of total carotenes were made to determine stability in the range −20° to 40° F. Five to 7 temperature conditions were investi-

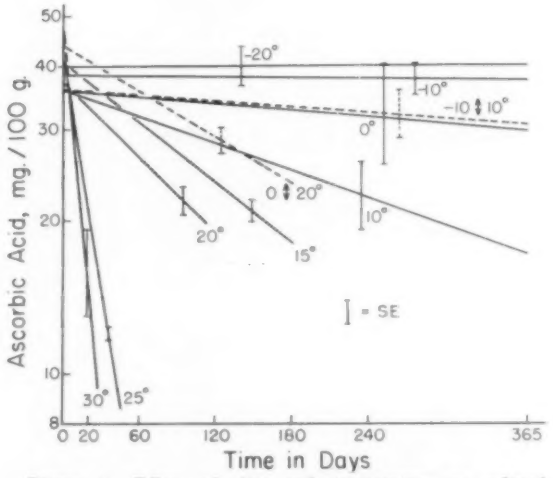

Figure 3. Effect of time and temperature on reduced ascorbic acid. Regression lines of ascorbic acid loss and standard error of estimate (SE) plotted on a semi-logarithmic graph, lot 6, Savoy variety.

gated for each lot. Some maximum time periods measured at a given temperature were 2 years at 0° and −20°, 1 year at 20°, and 7 days at 40° F. There was no significant change with time at any given temperature, which is in accord with the work of Bedford and Hard *(1)* and Burger *et al (8)*. All values for each lot were averaged and the SD calculated (Table 2).

Reflectance color. Measurement of color attributes with the Hunter Color and Color Difference Meter (HCM) at 10° intervals from 0 to 30° F, showed that change in color with time was complex. Although HCM *a*, saturation index, and hue angle showed reasonably consistent patterns of change with time and temperature, these functions did not change at constant rate during the periods tested as did percentage chlorophyll conversion. Saturation index changed relatively fast towards grayness or muddiness of green color during the first stages and much slower in the latter stages of deterioration. Hue angle, $\tan^{-1}$ b/a, changed irregularly at first before

TABLE 2

Average carotene retention in the range of −20° to 40° F

Lot No	1L	2C	3L	4C	5L	6L
Carotene, mg/100 g	5.1	4.8	4.9	3.4	4.9	5.9
SD mg/100 g	0.37	0.40	0.47	0.46	0.23	0.77
Number of observations	28	11	17	10	15	17

a consistent decrease occurred to indicate a change from green toward yellow as time increased at a given temperature. The HCM L data for 30° F showed little or no darkening of green color in contrast to a decided darkening in the early stages of deterioration at 0°, 10°, and 20° F and in the 20° F data of Boggs *et al (6)*; similar color changes were observed in green beans and peas. After an initial drop, the L values leveled off or increased.

Guerrant *(13)* made reflectance measurements on a 1 + 1 spinach-water slurry with a recording spectrophotometer from 400 to 700 mme. After 12 months at 10° F, the spinach color was darker as indicated by a marked lower reflectance in the green-yellow-orange region.

There was a high degree of correlation (Table 3) between percentage conversion of chlorophyll and

TABLE 3

Correlation of percentage conversion of chlorophyll with HCM functions [1]

Measurements correlated with chlorophyll conversion, %	Correlation coefficient		
	Lot 5	Lot 6	Lot 5 + 6
Log HCM a	−0.95	−0.94	−0.80
Log HCM $\sqrt{a^2 + b^2}$	−0.93	−0.85	−0.60
Log HCM $\tan^{-1} b/a$	−0.93	−0.88	−0.40

[1] Values for each holding time at 0°, 10°, 20°, and 30° F.

logarithmic values of HCM a, saturation index, and hue angle for individual lots. However, this high degree of correlation was lost when data for lots 5 and 6 were combined; lot 5 was yellow-green, whereas, lot 6 was blue-green. For general trends and intercomparison of HCM functions, see the graphs of Boggs *et al (6)* illustrating data obtained from spinach held 0 to 84 days at 20° F.

Relation of judges' results to objective measurements. In order to determine any association of objective measurements with color judgments for a wide range of temperature conditions and deterioration, 19 or 20 samples of each lot were scored in one series. Vivid green color similar to a labeled control was scored 1, and a very yellow or very grayed green was scored 5. Samples from 1 to 5 time periods for each of 6 or 7 temperature conditions ranging from −20° F to 30° F made up a color series for lots 5 and 6. Judges' scores (Table 4) show a high degree of correlation with chlorophll conversion, HCM a, and HCM $\sqrt{a^2 + b^2}$ over the wide range of color selected

TABLE 4

Correlation for judges' color scores with objective data [1]

	Correlation coef	
	Lot 5	Lot 6
Color scores with		
Chlorophyll, % conv	+.92	+.93
Ascorbic acid, mg/100g	−.87	−.86
HCM a	−.97	−.93
HCM $\sqrt{a^2 + b^2}$	−.96	−.91
HCM hue angle, $\tan^{-1} b/a$	−.82	−.75
HCM L	−.64	−.73

[1] Average values for all packages measured for each time-temperature condition. Does not include any material used for visual judgments.

for these series. Chlorophyll conversion ranged from 6 to 37%; when the latter condition was found, the flat-leaf portion was gray-green and the stems yellowish or brown.

To determine the amount of difference in objective values required for judges to distinguish between samples, 130 to 150 pairs were ranked and objective measurements made on the same material. Intercomparisons were made between samples held at several temperatures from −20° to 30° F and for different time periods at a particular temperature. Larger differences in chlorophyll were required for visual recognition of color differences in lot 5 than in lot 6, although similar differences were found for HCM a and $\sqrt{a^2 + b^2}$ (Table 5).

When chlorophyll conversion was above 21%, larger conversion differences were required for judges to distinguish between samples. Chlorophyll and HCM functions changed similarly up to 4 to 10% change in conversion, then HCM values (visual changes) changed less rapidly than chlorophyll. This difference in rates could account for the requirement of greater change in chlorophyll in order to see a visual difference above 21% conversion of chlorophyll. To find the time required for a visual change in color to occur on the basis of chlorophyll conversion, divide the average difference (Table 5) by the rate per day at a particular temperature (Figure 2). Thus, lot 5 would change visually after 350 days at 0°, 70 days at 10° or 7 days at 25° F.

First detectable flavor differences with respect to time, chlorophyll conversion, and ascorbic acid. Judges were specially trained and selected for flavor appraisal of lots 5 and 6. They were consistent with themselves when tested on deteriorated samples at the start and the end of a 14-month period; the latter samples had been preserved at −20° F. Time at each temperature when 75% of judgments concurred that samples had more off-flavor than material held continuously at −20° F are plotted against holding temperatures in Figure 4.

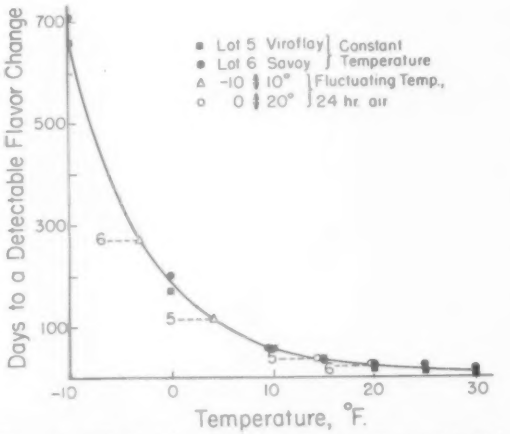

Figure 4. Effect of temperature on flavor. Days when 75% of judgments concurred there was a flavor change from −20° F controls.

TABLE 5

Chlorophyll and HCM differences required for 78 to 89% of judgments to indicate a difference in color between two samples[1]

	Chlorophyll conversion		HCM *a*		HCM $\sqrt{a^2+b^2}$		HCM, hue angle		HCM L	
Lot No	5	6	5	6	5	6	5	6	5	6
Average difference	+3.5	+0.8	−0.6	−0.5	−0.8	−0.6	−0.9	−0.2	−0.7	−0.9
S D	3.7	2.5	0.9	0.5	1.0	0.7	2.4	2.8	1.5	1.4
S E of average	0.6	0.3	0.14	0.07	0.16	0.09	0.4	0.4	0.24	0.20
Number of pairs[1]	43	53	43	53	43	53	43	53	43	53

[1] Pairs were ranked 1 and 2; 1 = more vivid or less yellow and 2 = more yellow or gray. The symbol + or − indicates increase or decrease of objective value with increase in rank.

A detectable off-flavor developed in 4 to 5 months at 0° F and there was a rapid decrease in time as temperature was increased until only a few days were required at 30° F. Bennett *et al (2)* found "three quality levels in flavor and color" in spinach due to different temperature effects at −20°, 0°, and 10° F when samples were compared after 4 months. Tinklin and Filinger *(15)* blanched spinach by 3 methods and reported changes in flavor, aroma, texture, appearance and ascorbic acid retention when held at 0° F for 1 to 6 months.

During the development of a detectable flavor change, the accompanying decrease in percentage chlorophyll ranged from 1.1 to 5.7 and the decrease in mg per 100 g of ascorbic acid ranged from 2.0 to 9.3 for 10 different temperature conditions, lot 5. For 7 temperature conditions, lot 6, the chlorophyll decrease ranged from 1.1 to 4.4 and ascorbic acid decrease ranged from 2.4 to 5.4. Time periods required for detectable flavor and visual color changes were about the same at a given temperature as determined by equivalent chlorophyll changes in respective samples.

Industry evaluation. Time series of 0°, 10° and 20° F samples with a −20° F control included were evaluated by industry panels for acceptability on the basis of color and appearance of barely thawed material. Time-temperature effects caused a marked change in acceptability and the general patterns of change with time were similar for different lots even when judged by different panel members (Figure 5). Additional data for lots 5 and 6, Table 6, showed that panel scores and per cent of panel giving score 3 (consumer complaint score) increased in direct proportion to the increase in chlorophyll conversion.

Hunter functions *a*, hue angle, and $\sqrt{a^2+b^2}$ decreased with decrease in acceptability, but the pattern of points was more scattered. Before there was much

TABLE 6

Industry panel scores and objective data

Lot No	Time days	Temp °F	Score	Score 3 %	Chlorophyll conversion %	Ascorbic acid mg/100 g	HCM values			
							L	$\sqrt{a^2+b^2}$	Hue angle	*a*
1L	control[1]	−20	1.0	0	6.9	44				
	120	0	1.0	0	7.9	43				
	240	0	1.1	0	8.9	43				
	365	0	1.5	0	10.8	30				
1L	120	10	1.5	0	12.3	34				
	240	10	2.0	9	16.8	30				
	365	10	2.7	73	20.1	40				
1L	14	20	1.0	0	11.8	41				
	42	20	1.8	0	16.4	37				
	56	20	2.4	36	21.1	36				
	112	20	2.7	73	31.6	29				
3L	control	−20	1.0	0	8.6	42				
	14	20	1.2	0	16.7	32				
	28	20	1.8	10	16.4	30				
	42	20	1.8	0	27.2	21				
	56	20	2.2	36	22.8	28				
	112	20	2.8	82	30.7	18				
5L	control	−20	1.0	0	8.4	47	24.7	10.1	142.6	8.0
	112	10	2.1	16	15.9	38	22.7	8.1	135.5	6.0
	240	10	2.2	30	23.8	28	21.6	7.0	136.2	5.1
	365	10	2.9	95	33.8	24	21.9	5.9	132.2	3.9
5L	42	20	1.3	0	16.6	38	22.8	7.8	138.2	5.8
	56	20	2.4	41	22.8	30	23.4	6.7	136.2	4.9
	112	20	2.5	51	32.8	23	24.5	7.2	132.2	4.9
6L	control	−20	1.0	0	6.6	40	21.4	8.8	146.5	7.3
	112	10	1.6	3	13.7	26	18.2	7.0	144.6	5.8
	240	10	2.4	43	20.4	22	18.1	5.8	139.5	4.4
	315	10	2.4	46	24.0[2]	19[2]	16.8	4.9	142.6	4.1
6L	42	20	1.4	0	15.7	29	18.6	6.8	145.2	5.7
	56	20	1.8	6	18.1	26	18.8	5.6	142.3	4.4
	112	20	2.6	59	26.7	20	18.8	5.0	139.9	3.8
	180	20	2.9	92	39.3	13	17.5	4.3	.....	2.9

[1] A control was placed in each temperature series. Lots 1 and 3 scored by 1 panel, and lots 5 and 6 scored by 2 panels and data averaged.
[2] Interpolated values.

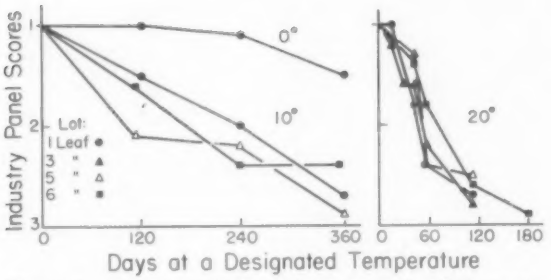

Figure 5. Industry evaluation of 0°, 10° and 20° F samples.
Score 1 = acceptable and high quality; score 2 = acceptable but not high quality; and score 3 = sufficiently poor to cause consumer complaint.
Lot 5 and 6 were judged twice (Albany and Seattle) and data averaged; other data were single evaluations.

increase in score above 1, ascorbic acid had decreased 10 to 20 mg/100 g. The data indicated that members of industry observed considerable deterioration in the quality of spinach held at 10° and 20° F. With minor exceptions, panel members gave a score of 3 to most lots held 8 months at 10° F or for 2 months at 20° F.

SUMMARY

Time-temperature conditions ranging from one day at 40° to two years at −20° F have been investigated for spinach with regard to changes in chlorophyll, ascorbic acid, carotene, reflectance color, visual color, and flavor. Significant deterioration did occur (except for carotene) above 0° F within periods of handling that may be experienced in industry practice. Measurable rates increased by a multiple of 10 for about each 15° rise in temperature between 0° and 25° F. This increase in rates was similar to the temperature effects on rates found earlier for peas and green beans.

Comparison of change-in-percentage chlorophyll converted to pheophytin in spinach with the change in peas and green beans, for example at 10° and 20° F, showed conversion in spinach to increase 1 to 2 times as fast as in peas and one-half to one-quarter as fast as in green beans. Loss of ascorbic acid in spinach (expressed as mg/100 g) was 1 to 2 times as much as found in green beans and peas under the same conditions.

Some time-temperature effects caused enough deterioration for industry panels to rate samples as "sufficiently poor to cause consumer complaint." Data emphasize a large variation among 10 oz packages of spinach and the necessity for adequate sampling for quality evaluation.

Acknowledgment

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An Accelerated Oxidation Method for the Estimation of the Storage Life of Frozen Seafoods[a, b]

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COLD STORAGE of many seafood products often leads to losses in quality and fresh flavor. Prolonged freezer storage coupled with the variable temperatures frequently encountered in commerce promote these deleterious changes. In addition, the large proportion of unsaturated lipids common to fishery products makes them particularly vulnerable to attack by atmospheric oxygen. This autoxidation reaction, oxidative rancidity, is characterized by the formation of hydroperoxides. The decomposition of these hydroperoxides yields carbonyls, the compounds responsible for the off-flavors and odors that taste panels classify as "painty," "fishy," or "rancid."

The problem of lipid deterioration by oxygen has been the subject of several excellent reviews by Morris *(13)*, Watts *(21)*, and Lea *(11, 12)*. The subject of seafood autoxidation was discussed by Yu and Sinnhuber *(22)*. They used the 2-thiobarbituric acid (TBA) method as a measure of the extent of oxidative rancity, applying this test to the intact sample, without extraction. Results were expressed as TBA number or mg of malonaldehyde per 1000 g of sample *(16, 22)*.

Accelerated aging tests to promote oxidation of fats, oils and food products are widely used in the food industry to evaluate processing procedures and predict probable shelf life. Reviews by Riemenschneider *(5)*, Lea *(10)*, and the Committee on Food Research, QMC 17-7 *(6)*, discuss the usefulness of various accelerated tests. Gearhart and Stuckey *(9)* proposed an ASTM Bomb Method and compared it with the Active Oxygen Method and the Schaal Oven procedures. These methods all employ exposure to air or oxygen at normal or increased pressure and controlled temperature to accelerate oxidation.

Lea *(10)* suggested that the area of surface exposed to air would have a considerable effect on the rate of oxidation. In early incubation tests filter paper was used as a support for fat to increase the surace area. Eckley *(6)* reported an oxygen absorption method for measuring the induction period. In this method sand is used as a support to increase surface area. In a very recent investigation by Bishov, Henick and Koch *(1)* carboxymethylcellulose is used as a support to evaluate antioxidants in dehydrated foods.

The authors, as of the time of this report, are not aware of any method suitable for accelerating the aging of frozen seafoods. The long storage period required to evaluate frozen fishery products, the product to product variation, and variation within the catch—all combine to make storage tests time-consuming and oftentimes devoid of any useful data. For example, antioxidants although known to be useful stabilizers of fat and widely used in other foods to extend their shelf life, are not used by the fishing industry. Perhaps one reason for the lack of use of antioxidants is the inability to give a clear cut demonstration of their value. An accelerated oxidation method for frozen seafoods would be a useful tool to evaluate processing procedures such as the choice of antioxidants, proper storage temperatures, and pre-handling and processing conditions.

Diatomaceous earth such as Celite which has a surface area from 4 to 6 square meters per gram offers interesting possibilities as a support medium not only for fats and oils but also for seafoods.

The acceleration oxidation method described in this paper uses the surfactant, Celite, as an inert support for frozen fish or fat to increase enormously the surface area. This increased exposure to air permits greatly increased oxidation even at sub-freezing temperatures. The effect of temperatures and their fluctuations during storage, action of antioxidants, and the relation of temperature to oxidation rate in frozen seafoods is described. The TBA test is conducted directly on the fish-Celite samples. This procedure is also proposed as a method for the accelerated autoxidation of fats and oils and several advantages are claimed for its use.

EXPERIMENTAL

Reagents. *NDGA antioxidant solution.* Ten per cent nordihydroguaiaretic acid (NDGA), 20% butylated hydroxyanisole (BHA) and 6% citric acid dissolved in edible oil and propylene glycol (Nordigard Corp.).

Propyl gallate (PG) (Eastman Chemical Products, Inc.) *antioxidant solution.* Stock solution containing 1 mg/ml prepared by dissolving 100 mg of PG in absolute ethanol and diluting to 100 ml.

Quercetin (Q) (Weyerhaeuser Co.) *antioxidant solution.* Recrystallized two times in an acetone-water solution and dried over KOH in a vacuum. Stock solutions were prepared as with PG above.

Diatomaceous silica. Celite Analytical Filter-Aid and Celite 319. (Johns-Manville Corp.).

Freon 11 (Matheson Co.) *trichloromonofluoromethane.*

[a] Approved for publication as Technical Paper No. 1328 by the Director of Oregon Agricultural Experiment Station as contribution of the Department of Food and Dairy Technology.

[b] Presented at 20th Annual Meeting of the Institute of Food Technologists, San Francisco, May 17, 1960.

[c] From a thesis by Robert E. Palmateer submitted in partial fulfillment for the degree of Master of Science.

Lard. Steam extracted lard was obtained from a commercial source. The lard was packed at 158° F (70° C) in 307 x 409 "C" enamel cans and held at 0° F (−18° C) until used. The lard had the following properties: Free fatty acid content, 0.55% (expressed as oleic acid); iodine number (Hanus), 57.53; stability by the Active Oxygen Method, 8.8 hr to reach 125 milliequivalents peroxide, and after ignition the residue from 25 g of lard gave a negative copper test with phosphomolybdic acid *(7)*.

Preparation of sample. Fresh rockfish fillets *(Scorpaenidae sp.)* were purchased locally for each treatment. Approximately 2 lb of sample were passed through a meat grinder modified to prevent iron contamination by chrome plating the internal parts and utilizing a Formica face plate. After being ground, the sample was well mixed, quartered, and the alternate quarters discarded. This was repeated three times after which a 10–20 g random sample was removed.

Preparation of surfactant. A diatomaceous silica, Celite Analytical Filter-Aid was selected because it is commercially available and uniform in quality. Previous tests have shown that this product does not interfere with or affect either the 2-thiobarbituric acid (TBA) reaction or peroxide determinations. Manufacturer's specifications of this and similar diatomaceous silica products point out their large surface area per unit of weight (*i.e.*, from 4 to 6 sq m per g). Chemical analysis, for the presence of trace metals which would influence the oxidation reaction revealed appreciable amounts of iron. Purification and removal of the iron was accomplished by reacting the surfactant with aqua regia for 48 hr. The surfactant was then transferred to a large percolator and washed with distilled water, using suction, until a negative chloride test was obtained with silver nitrate. The water was removed at the pump and the product dried in an oven at 212° F.

Method of mixing surfactant with seafoods. A random sample (10.00 g) of the seafood to be tested was obtained as previously described and mixed with purified Celite Analytical Filter-Aid, (30.00 g), by thoroughly grinding the sample in a No 6 mortar with a small amount of surfactant. The remainder of the filter-aid was added and blended into the original to yield a final proportion of 1:3. Standardization of the mixing procedure as to time and temperature is important to achieve uniformity. The mixture was next passed through a 20-mesh nylon screen and placed in suitable containers for storage.

Method of mixing surfactant with fat. A uniform dispersal of fat on surfactant was obtained by the following procedure: 20.00 g of lard were quantitatively transferred from the weighing beaker to an 800 ml beaker by the use of three 25 ml portions of redistilled Freon 11. Twenty g of purified surfactant was added with stirring until a homogenous mixture was obtained. The Freon was evaporated by stirring the mixture over a warm water bath (120° F) until no lumps remained and a fine powder resulted. Complete evaporation of the solvent was unnecessary since any traces were evaporated in the constant temperature oven.

Chemical determination of oxidative rancidity. The 2-thiobarbituric acid (TBA) method of Yu and Sinnhuber *(22)* was adopted with only minor modifications. A crystal clear solution was achieved after centrifuging for 10 min at 1800 rpm, eliminating the extraction procedure with petroleum ether. The results are expressed as TBA number (mg of malonaldehyde per 1000 g of sample) by multiplying the $E^{1\%}_{1cm}$ at 535 mme by the constant 46 *(17)*.

The Peroxide Value, Cd 8-53 *(1)* was used in determining the stability of a lard substrate mixed with antioxidants and purified Celite Analytical Filter-Aid. The following modifications were made: A 0.01 N-sodium thiosulfate solution was used for titrations on a 2.000 g sample containing 1.000 g of lard; it was found necessary to swirl the flask continuously for 1 min, prior to the addition of potassium iodide, to break up the lumps present.

PROCEDURE NOTES AND RESULTS

Effect of purification of surfactant on the accelerated autoxidation of frozen fish. To determine the need for purification of the surfactant and the effect of metal (expressed as iron) on the accelerated antioxidation test, the following experiment was conducted. A random sample of the fresh, ground rockfish fillets was divided into 3 portions and individually mixed with the 3 diatomaceous silica products selected, in a mortar using the prescribed procedure. The products used were: Celite 319; Celite Analytical Filter-Aid; and the purified Celite Analytical Filter-Aid. The latter material was purified, as previously described, by treatment with acid. The iron content of Celite 319 was 1.0%–1.5%; of Celite Analytical Filter-Aid, 0.2%; and the purified product contained less than 2 ppm *(8, 18)*.

The samples were placed in one ounce screw-cap jars, after sifting through a 20-mesh nylon screen, and stored at 0° F for subsequent TBA determinations.

Figure 1 shows the effect of purification and the correlation which exists between the removal of metals (iron) on the autoxidation test. The need for purification of the analytical grade Celite is demonstrated.

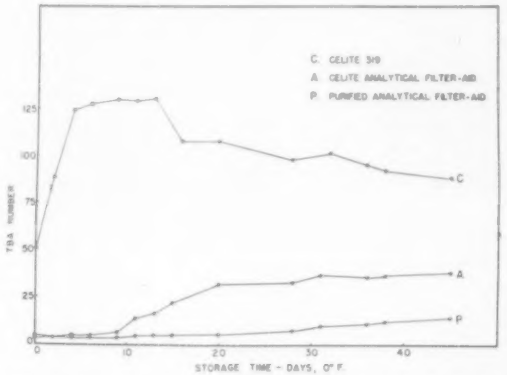

Figure 1. The effect of purification of surfactant on the accelerated autoxidation of frozen rockfish.

This procedure could be used to study the catalytic oxidation action of metals, the pro-oxidant effect of hemoglobin, and the function of metal deactivators or synergists in frozen seafoods.

Influence of temperature on the development of oxidative rancidity. Use of low temperature storage is recommended by technologists for the maintenance of good quality in frozen seafoods. They emphasized the importance of storing fishery products at 0° F or below and demonstrated that the necessity for low temperature storage increases as the length of the storage period is increased *(20)*.

Quality determinations for stored, frozen seafoods and the evaluation of treatment and processing variables is often very difficult because of the wide package-to-package variation that frequently occurs.

The experiment described below demonstrates, in a short time, the effect of storage temperature on the development of oxidative rancidity in frozen fish.

Fresh rockfish fillets were purchased from a local source for this experiment. A random sample was obtained and mixed with purified Celite as previously described. Fish-Celite samples were weighed into 1 oz

screw-cap glass jars for storage at 0°, 10°, and 20° F. Each jar contained approximately 1.5 g and served as an individual random sample for the TBA reaction.

In Figure 2 the effect of storage temperatures on the rate of autoxidation of a surfactant-fish mixture is demonstrated. The rate of the oxidative reaction is approximately doubled for an 18° F rise in temperature. This observation confirms the work reported by

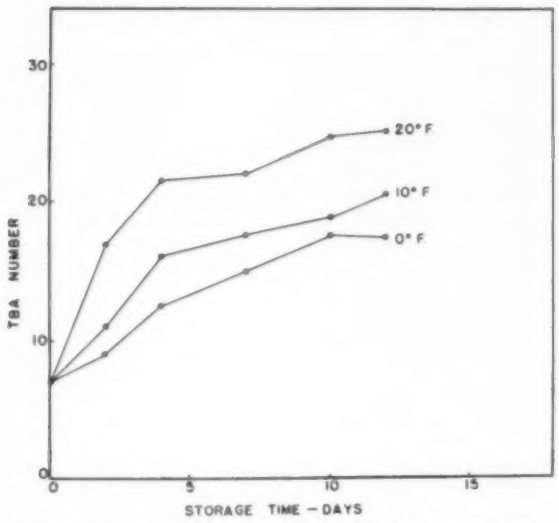

Figure 2. The effect of storage temperature on accelerated autoxidation of frozen fish.

Lea *(10)*. It seems apparent that by this accelerated procedure, it may be possible to evaluate storage variables and various additives in a matter of days. Previous experince has shown that weeks or even months would be necessary to achieve a similar state of oxidation with whole or even ground fillets.

Effect of fluctuating temperatures on the autoxidation of seafoods. Pottinger *(14)* emphasized the need for keeping frozen seafoods at a constant storage temperature, pointing out that to achieve this ideal condition is difficult. He listed some of the factors that were responsible for causing temperature fluctuations: using storage rooms for freezing; overloading the refrigeration equipment; power failure; equipment breakdowns; improper setting of control devices; transferring frozen products from one storage room to another; and transporting frozen products from producer to consumer. Considering the number of opportunities for the temperature to change, it is probable that some products undergo a series of fluctuations in temperature before reaching the consumer.

The effect of several temperature fluctuations on the extent and rate of outoxidation was demonstrated by using this accelerated autoxidation procedure. Temperature fluctuations similar to those encountered in commercial handling were simulated by the following procedures: 36 one-pound cartons of commercially produced, fresh-frozen rockfish fillets were obtained from a commercial producer. Initial TBA determinations were made and a similar sample was presented to a trained panel for organoleptic evaluation. The results in each case indicated a relatively fresh sample. The remaining cartons were evenly divided into 4 lots (0x, 1x, 2x, and 3x). Lot 0x (control) was held at 0° F. Lot 1x, 2x, and 3x were placed in a 35° F cool room and held for 12 hr, during which time the internal temperature of all fillets uniformly increased to a maximum of 29° F as recorded by a copper-constantin thermocouple. To assure stabilization of temperature, samples were rapidly cooled to 0° F and held at that temperature for 24 hr between each fluctuation period. Lot 1x was held at 0° F, lot 2x and 3x were returned to the cool room and subjected to another 12 hr fluctuation in temperature. After stabilization at 0° F lot 3x was given a third and final treatment. After this third fluctuation period, 2 cartons were randomly selected from each lot, ground and mixed with the purified diatomaceous silica as described previously. Each lot was then stored in an 8-oz, screw-cap, glass jar and held at 0° F for the accelerated oxidation test. Rancidity measurements were made at intervals by the TBA methods.

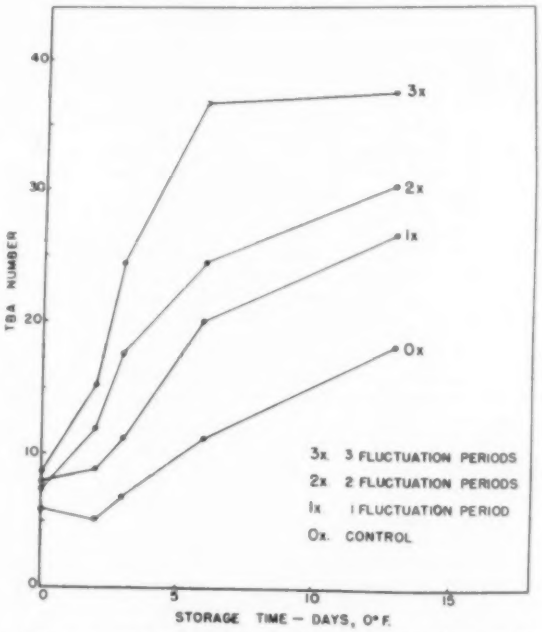

Figure 3. The influence of temperature fluctuations on the accelerated autoxidation of frozen rockfish.

The results (Figure 3) demonstrate the effect of fluctuations in storage temperature on the autoxidation of fat. The degree of oxidative rancidity increases with an increase in the number of fluctuation periods. This rapid increase in rate of autoxidation is in direct contrast with results shown in Table 1, where little change is seen in TBA numbers of commercial cartons from the same lots. It seems apparent that the accelerated autoxidation procedure can be

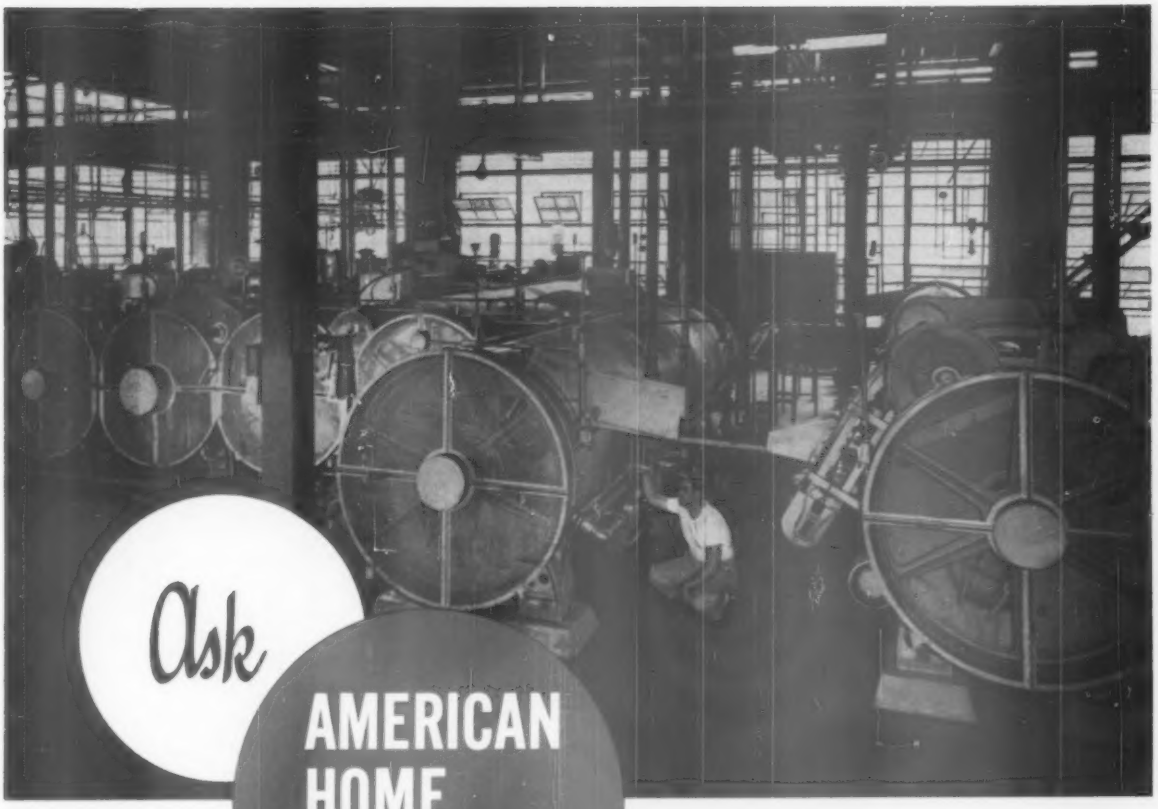

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TABLE 1

Autoxidative changes in commercial packaged frozen rockfish fillets subjected to fluctuations in storage temperature

Lot	0 Day TBA No	5 Days TBA No	20 Days TBA No
Control	1.14	1.24	1.21
1x		1.15	1.20
2x		1.20	1.43
3x		1.38	1.56

used to evaluate various processing variables in a very short time, not only in seafoods but in frozen vegetables and precooked frozen foods.

Influence of antioxidants on the accelerated autoxidation test. The need for antioxidants to protect frozen seafoods and thereby increase their storage stability has been the subject of considerable research and development *(2, 3, 15, 18, 19)*. In spite of the advantages shown by the application of antioxidants to other foods, they have not received a very enthusiastic reception by the fisheries industry. This lack of acceptance in spite of the great need, may be due in part to the difficulty of application and the costs involved. Sample variations and lengthy storage requirements often make a clear-cut evaluation of an antioxidant treatment a formidable task.

The accelerated autoxidation procedure was used to demonstrate the effect of an antioxidant solution on frozen salmon.

Fresh silver salmon steaks *(Oncorhynchus kisutch)* were prepared in the same manner as the rockfish fillets, after carefully removing the skin and bones. The sample was divided, 100 g thoroughly mixed with 70 mg of propylene glycol to serve as the control, the other half carefully mixed with 70 mg of the NDGA solution to yield a synergistic mixture of 0.007% NDGA, 0.014% BHA and 0.0042% citric acid concentrations on a final weight basis. A 1:4 ratio of sample to surfactant was used in this case, and mixed in a mortar under standardized conditions of time and temperature. Storage was in 1-oz screw-cap, glass jars at 0° F.

The results (Figure 4), clearly demonstrate the effectiveness of the antioxidant solution used. It can also be noted that less than 30 days is needed to evaluate the stabilizing effect of the antioxidant instead of the several months usually required. The high TBA number obtained for the rancid salmon tissue is apparently due to the high fat content and the highly unsaturated fats which are present.

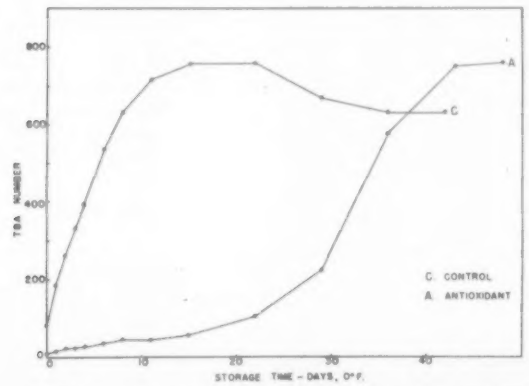

Figure 4. The effect of an antioxidant on the accelerated autoxidation of frozen salmon.

An accelerated autoxidation method for fats and oils. *Evaluation of antioxidants.* This experiment was designed to study the autoxidation of lard supported on purified Celite and the effect of the antioxidants, propyl gallate and quercetin on the stability of lard. These antioxidants, in a small amount of absolute alcohol, were added to lard to make a concentration of 0.0005 M. The same amount of absolute alcohol was used in the control sample. The samples were kept in a constant temperature oven at 140° F. At intervals, a portion of the sample was removed for peroxide determination *(1)*. The contents were stirred thoroughly prior to sample removal. Celite did not interfere with the peroxide determination.

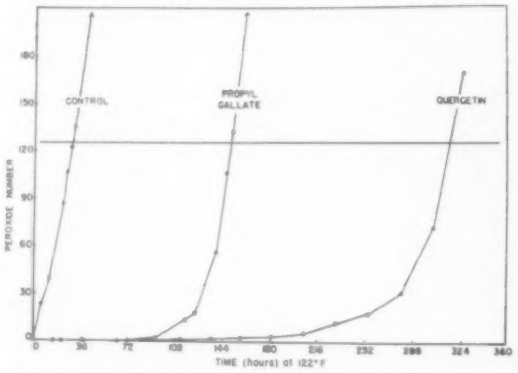

Figure 5. The effect of autoxidants on the accelerated autoxidation of lard.

The results of this experiment are shown in Figure 5. It can be seen that 30 hr were required for the control to reach a peroxide value of 125 me per 1000 g of sample (lard), which is the stability value according to the AOM procedure *(1)*. Propyl gallate and quercetin reached this value after 153 and 317 hr, respectively. The order of greater stability of quercetin over propyl gallate is a reversal of results usually obtained by the AOM procedure. A possible explanation is that since mole concentrations of antioxidants were used, a greater amount of quercetin was present than propyl gallate. This factor does not entirely explain the superiority of quercetin over propyl gallate. A possible additional explanation might be that the surfactant may contain sufficient iron to act as a pro-oxidant in lard. Quercetin, owing to its metal-chelating activity, may render the iron inactive and thereby extend the induction period. Confirmation of this observation is planned.

SUMMARY

An accelerated autoxidation procedure for frozen seafoods and fats and oils is presented. This method depends on the use of purified diatomaceous silica

(Celite) as a support for the material to be tested. The great increase in surface area that results permits autoxidation to proceed at an accelerated rate even at sub-freezing temperatures.

The effect of temperature on the rate of autoxidation was determined as well as the effect of fluctuating temperatures on the storage of frozen seafoods. The application of this procedure to evaluate antioxidants is demonstrated.

The proposed method is useful for the accelerated oxidation of fats and oils. The effect of the antioxidants, quercetin and propyl gallate on the stability of a lard substrate is shown by this method. The surfactant, Celite, does not interfere with the peroxide or TBA methods for the determination of oxidative rancidity.

The possible use of this accelerated autoxidation method for the accelerated storage of frozen vegetables and other foods is suggested.

ADDENDUM

Dr. C. H. Lea has advised us of the work by F. J. Honn, I. I. Bezman and B. F. Daubert entitled the "Autoxidation of Drying Oils Absorbed on Porous Solids" published in the *J. Am. Oil Chemists' Soc.*, 28, p. 129–133 (1951). This excellent paper, which was inadvertently overlooked by us, describes the use of silica gel as a dispersion medium for the autoxidation of soybean oil. Rate studies and critical oil to media ratios were determined.

Acknowledgment

The authors wish to thank the following companies for the reagents used in this study: Nordigard Corp., Chicago, Illinois, (NDGA antioxident solution); Eastman Chemical Products, Inc., Kingsport, Tenn. (Tenox PG); Weyerhaueser Co., Longview, Washington (Quercetin); and Johns-Manville Corp., Celite Division, New York, N. Y. (Celite 319 and Celite Analytical Filter-Aid). Our thanks are also extended to James Kindred and William Dieters of the Astoria Seafoods Co., Astoria, Oregon, for furnishing many of the samples use, and to Victor Horgan and William Elders of Sebastian-Stuart Company, Astoria, Oregon for packaging some of the experimental samples. The very helpful assistance of Duncan K. Law of the Seafoods Laboratory of Oregon State College at Astoria, Oregon in procuring samples, and of David L. Crawford of the Department of Food and Dairy Technology, Oregon State College, in evaluating the lard substrate is gratefully acknowledged.

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Equilibrium Relative Humidity (ERH) Relationships of Fruit Juice and Custard Powders

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(Manuscript received April 18, 1960)

MEASUREMENT OF Equilibrium Relative Humidity (the storage relative humidity at which a product will neither gain nor lose in weight) is of considerable value in a study of the packaging behaviour of dehydrated foods, and particularly so in the case of hygroscopic materials. This value is influenced considerably by the original moisture content of the product itself. The humidity moisture equilibrium curve or the Sorption Isotherm depicts the equilibrium relationship between the moisture content of the product and the RH of the atmosphere immediately surrounding it.

Various methods have been employed for the measurement of Equilibrium Relative Humidity (ERH). These are based on measurement of water vapour pressure by manometer *(1)*, direct measurement with electric hygrometer *(4, 7, 9)*, mathematical formulae *(3, 4)*, Wink's weight equilibrium method *(11)* and the graphical interpolation method *(5, 6)*. Of these, the last two methods are quite simple to follow and give reliable data. The ERH data of some important fruit juice powders and custard powder, recently developed in these laboratories, were determined by these two methods and are presented in this paper. ERH data for garlic powder has recently been reported from this laboratory by adopting a similar procedure *(8)*.

EXPERIMENTAL

Mango, banana, guava, and orange juice powders and mango custard powder (Mangotine) were employed for ERH studies. Mango, guava, and orange juice powders were prepared by vacuum shelf drying according to a process developed in our laboratories *(2)*. Banana powder was prepared from ripe *Pachabale* variety, using an atmospheric double drum drier. The dried flakes were powdered rough, sieved, and preserved in hermetically sealed tins. Mango custard powder was prepared by atmospheric drum-drying of strained Badami mango pulp containing added corn starch, sugar and skim milk powder, and sodium bicarbonate to neutralise part of the acidity and bring the pH to 5.4–5.6. This dried product, which was powdered and sieved, was intended to be used as a strained baby food after reconstitution into a smooth paste with warm water or milk. The fruit juice powders as well as the custard powder were hygroscopic and were therefore packed in closed containers soon after preparation and used in the subsequent experiments. The proximate composition of the products is given in Table 1.

Moisture content. The moisture content of the sample was determined by drying a known weight of the material (3–4 g) for 6 hr at 70° C in a vacuum oven at 26 in vacuum.

Equilibrium Relative Humidity value. This was determined by two methods: (a) graphical interpolation method and (b) weight equilibrium method.

(a) *Graphical interpolation method.* One hundred gram lots of each of the fruit powders were conditioned to different moisture levels by exposing them for varying periods to high humidity. In the case of each powder, 5-gram lots were weighed into tared Petri dishes (dia 2 in), spread uniformly, and the dishes kept inside desiccators containing sulphuric acid solutions of different strengths to maintain a complete range of relative humidities ranging from 0 to 98% *(5, 10)*. The desiccators were kept in the balance room at a temperature of 25° C. The dishes were weighed at the end of 1 hr and the increase or decrease in the weight of the sample noted. From these changes in weight, graphical interpolation isotherms were plotted and the point of intersection of these with the Zero base line was taken as the ERH value of the particular sample. From the values thus obtained, the moisture equilibrium curve was drawn. Typical interpolation isotherms in the case of mango, mango custard and banana powders only are given in Figures 1, 2 and 3.

TABLE 1

Proximate composition of fruit juice powders and custard powder

Particulars	Mango powder	Banana powder	Guava powder	Orange juice powder	Mango custard powder (Mangotine)
Moisture, %	1.41	2.75	1.05	1.88	1.97
Ash, total, %	0.30	3.90	0.24	0.88	2.68
Ether extract, %	0.21	0.12	0.10	0.19	0.17
Protein (N x 6.25), %	0.40	4.45	0.34	1.32	7.76
Acidity, as anhydrous citric acid, %	0.49	1.19	0.23	3.20	1.16
Sugar, reducing, as invert sugar, %	6.11	67.51	4.14	10.0	19.67
Sugar, total, as invert sugar, %	94.36	72.63	93.75	79.39	66.76
Crude fibre, %	0.32	1.56	1.66	0.12	1.68
Carbohydrates other than sugars (by difference), %	2.71	13.40	2.63	13.02	17.82

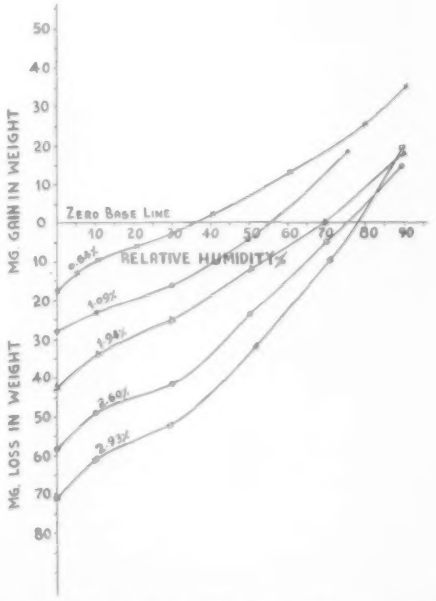

Figure 1. Graphical interpolation isotherms for mango powder (1 hr test period at 25° C).

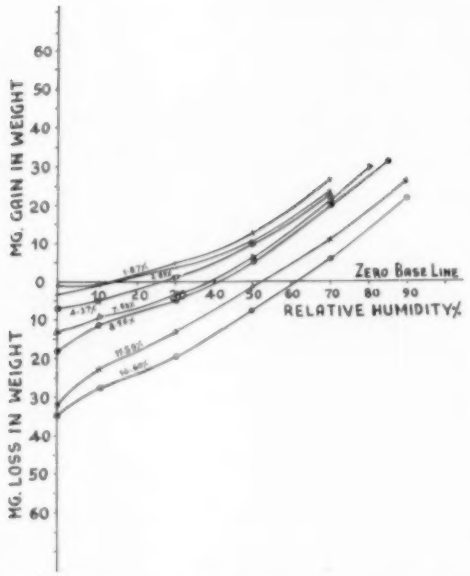

Figure 2. Graphical interpolation isotherms for mango custard powder (1 hr test period at 25° C).

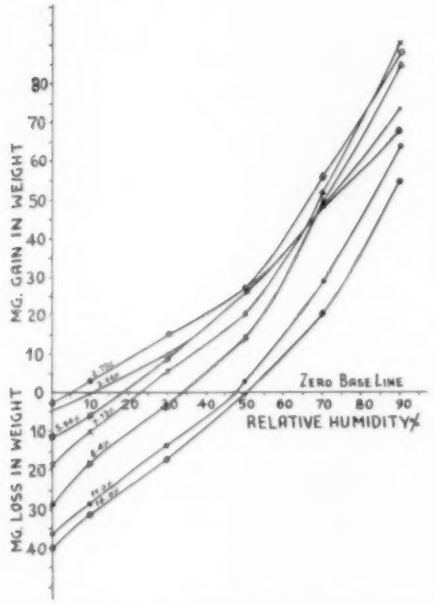

Figure 3. Graphical interpolation isotherms for banana powder (1 hr test period at 25° C).

(b) *Wink's weight equilibrium method.* Five-gram lots of the powder were weighed into Petri dishes (dia 2 in), spread uniformly and exposed to different relative humidities, ranging from 0 to 98% at 25° C, inside desiccators containing sulphuric acid solutions of different strengths, or saturated solutions of different salts having definite relative humidities at 37° C *(11)*. The gain or loss in weight of the sample was determined at the end of 1, 2, 3, 4, 5 and 6 hr and thereafter at intervals of 24 hr up to 360 hr, with a view to determine the moisture equilibrium of the product at 25° C. In the case of 37° C, the dishes were weighed at intervals of a fortnight up to 75–120 days, to observe the changes in moisture uptake and also any adverse changes in the product such as caking, discolouration, mould growth, etc, and thus get an idea of the critical point and other storage characteristics of the product. When there was no further loss or gain in weight of the product, the equilibrium moisture content of the product was calculated

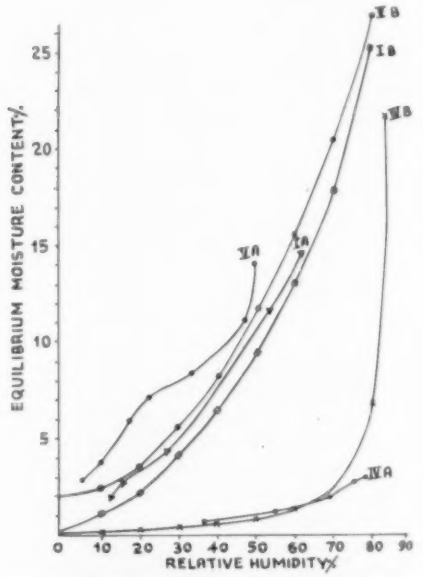

Figure 4. Humidity moisture equilibrium curves for mango custard powder, mango powder and banana powder by graphical interpolation and weight equilibrium methods (at 25° C).

Graphical Interpolation Method: IA—Mango Custard Powder; IVA—Mango Powder; VA—Banana Powder.

Weight Equilibrium Method: IB—Mango Custard Powder; IVB—Mango Powder; VB—Banana Powder.

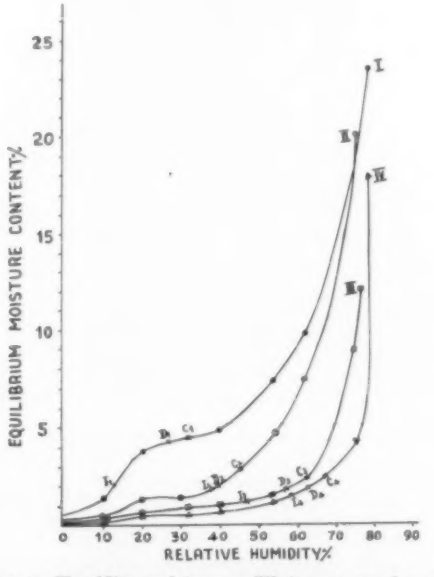

Figure 5. Humidity moisture equilibrium curves for mango, orange and guava powders and mango custard powder (at 37° C).

I—Mango Custard Powder; II—Orange Juice Powder; III—Guava Powder; IV—Mango Powder.

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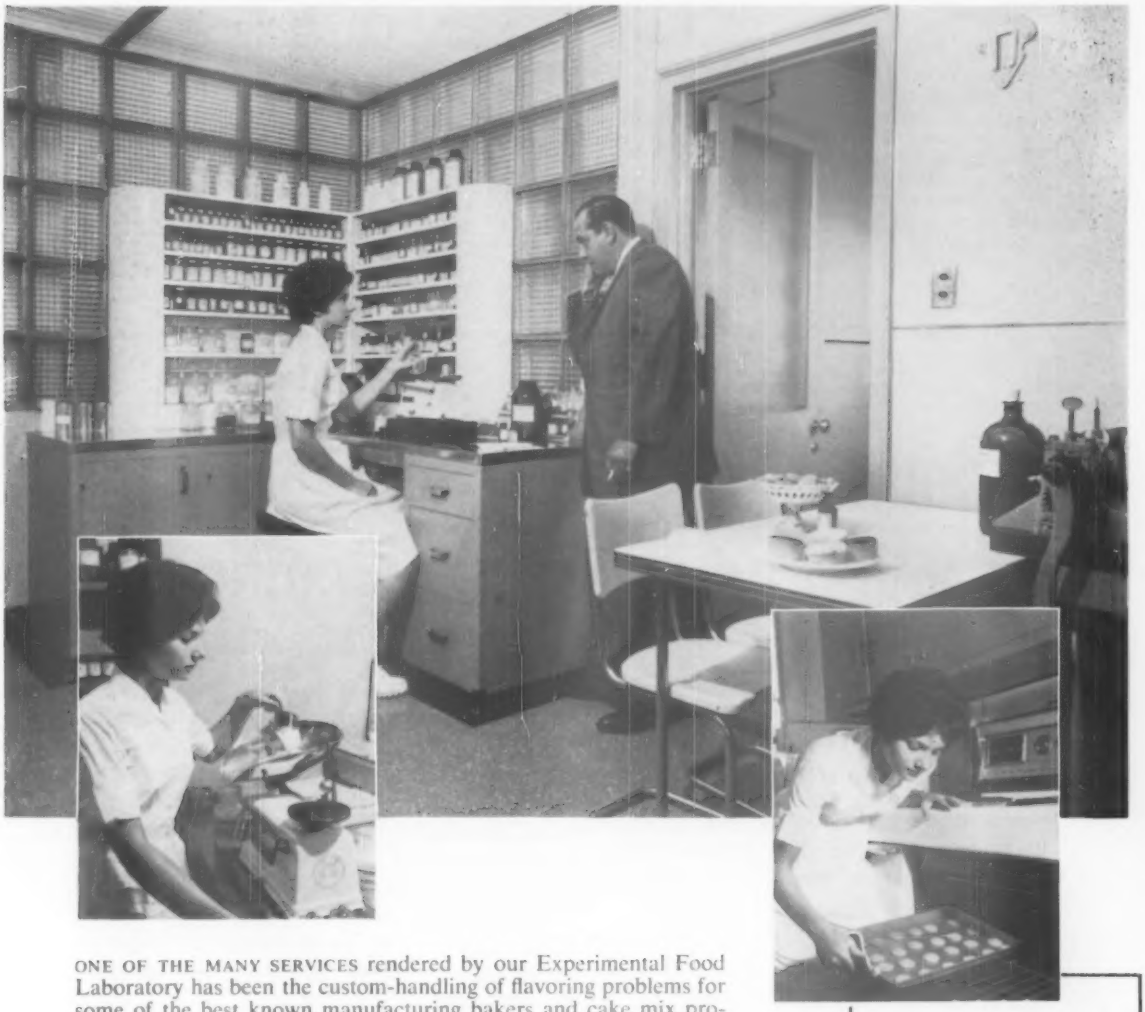

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TABLE 2

ERH for mango powder (0.64% moisture)

At room temperature (25° C)				At 37° C		
Graphical interpolation method		Weight equilibrium method		Weight equilibrium method		
Equilibrium moisture content	ERH	Equilibrium moisture content	ERH	Equilibrium moisture content	ERH	Remarks
%	%	%	%	%	%	
0.64	36.5	0.0	0.0	0.0	0.0	Product was good and free-flowing. Yellow colour. No change in colour.
1.09	55.5	0.09	10.0	0.06	10.0	Product was good and free-flowing. Yellow colour. No change in colour.
1.94	69.0	0.22	20.0	0.48	20.41	Product was good and free-flowing. Yellow colour. No change in colour.
2.60	75.5	0.34	30.0	0.42	31.9	Product was good and free-flowing. Yellow colour. No change in colour.
2.93	78.0	0.51	40.0	0.56	40.2	Product was good and free-flowing. Yellow colour. No change in colour.
		0.81	50.0	1.08	53.7	Product was good and free-flowing. Yellow colour. No change in colour.
		1.30	60.0	1.79	61.8	Product had faded in colour and was slightly sticky.
		2.22	70.0	4.13	75.1	Product was moist and cake formation was observed. Colour had slightly changed into brown. Mould appeared after 58 days.
		6.82	80.0	17.94	79.1	Product was moist and had turned into a liquid which was brownish in colour. Mould appeared after 23 days.
		23.0	90.0	44.35	91.1	Product had turned into a liquid which was brownish in colour. Mould growth appeared after 10 days.

from the data, making use of the original moisture content of the sample taken and the amount of moisture gained or lost during equilibration. The moisture equilibrium curves were then drawn. On these curves, the initial point (I), the danger point (D) and the critical point (C) were marked (Figure 5). The initial point represents the moisture content and the ERH of the product as prepared, and the critical point is the stage at which the product just becomes lumpy. A point, which is of 5% lower RH, is designated as the Danger Point. The portion of the curve between the two points D and C is generally arbitrarily defined as the safety range (SR) *(6)*. The portion of the curve between the points I and D may be defined as the safety margin (SM) as a helpful guide in the packaging of the material. It is desirable that the package adopted should not permit the product to reach this danger point, so that there may be a safe margin of error. From these considerations the fruit juice powders are highly hyroscopic and require stringent packaging conditions.

RESULTS AND DISCUSSION

Data regarding the ERH for mango, banana, and mango custard powders by the graphical interpolation method and also by the weight equilibrium method at

TABLE 3

ERH for banana powder (2.75% moisture)

At room temperature (25° C)				
Graphical interpolation method		Weight equilibrium method		
Equilibrium moisture content	ERH	Equilibrium moisture content	ERH	Remarks
%	%	%	%	
2.75	5.0	2.09	0.0	Free-flowing; light brownish yellow in colour.
3.66	10.0	2.42	10.0	
5.94	17.5	3.49	20.0	
7.13	22.5	5.50	30.0	
8.40	34.0	8.08	40.0	
11.0	47.5	11.46	50.0	
14.0	50.0	15.62	60.0	
		20.25	70.0	
		26.89	80.0	
		34.93	90.0	
			95.0	Mould growth after 3 days.

TABLE 4

ERH for mang ocustard powder (0.94% moisture)

At room temperature (25° C)				At 37° C		
Graphical interpolation method		Weight equilibrium method		Weight equilibrium method		
Equilibrium moisture content	ERH	Equilibrium moisture content	ERH	Equilibrium moisture content	ERH	Remarks
%	%	%	%	%	%	
1.87	12.5	0.24	0.0	0.50	0.0	Product was good and free-flowing. Original orange yellow colour. Colour not affected.
2.89	15.0	1.11	10.0	1.32	10.0	Product was good and free-flowing. Original orange yellow colour. Colour not affected.
4.37	27.0	2.10	20.0	3.71	20.4	Product was good and free-flowing. Original orange yellow colour. Colour not affected.
7.88	38.5	4.13	30.0	4.36	31.9	Product was good and free-flowing. Original orange yellow colour. Colour not affected.
8.56	41.5	6.40	40.0	4.75	40.2	Product had faded in colour. Caking was observed.
		9.40	50.0	7.31	53.7	Product was slightly brownish in colour and cake formation was observed.
11.59	53.5					
		12.90	60.0	9.60	61.8	Product had faded in colour. There was cake formation.
14.60	61.5	17.60	70.0	18.04	75.1	Product was brown and caky. Mould appeared after 30 days.
		25.2	80.0	23.47	79.1	Product was brown and caky. Mould appeared after 20 days.
		35.4	90.0*	24.56	91.1	Product was brown and caky. Mould appeared after 8 days.
			95.0*			

* After 8 days, mould growth.

25° C as well as at 37° C are given in Tables 2, 3, and 4, respectively. It will be seen from Figure 4, giving typical curves at 25° C for mango, mango custard, and banana powders, that the weight equilibrium method gives more precise data than the graphical interpolation method. The two methods, however, give fairly similar isotherms except in the case of banana powder, indicating thereby the possibility of utilizing the quicker graphical method for the determination of the approximate ERH of the products. In the case of banana powder the isotherm by the graphical method differs significantly from that by the weight equilibrium method. The difficulty experienced in determining accurately the moisture content of the banana powder which had been prepared from pure pulp of the ripe fruit, may be partly responsible for the scatter in the values for moisture content of the powder in the graphical interpolation method. The condition of the powder during prolonged storage in regard to lumpiness, caking, mould growth, etc, is recorded in the remarks column. It will be seen from Figure 5 that the humidity moisture equilibrium curves for guava and mango powders are very similar, whereas the curves for orange juice and mango custard powders are significantly different from these and from each other. There is a wide variation as regards the range of ERH in relation to increase in moisture content in the case of the 4 powders. Thus mango and guava powders pass through a much wider span of ERH's before their moisture content increases by even 1–2%, than orange juice powder and mango custard powder. Mango custard powder undergoes a significant increase in moisture content along with the increase in ERH. The increase in moisture content above 50% RH is steep in the case of all the 4 products, thus bringing them into the category of hygroscopic substances. Data regarding the initial, critical, and danger points for moisture content and RH for the 4 products are summarised in Table 5. The condition of the product at various moisture levels is described briefly in the remarks column.

It will be seen that the packaging of mango, guava and mango custard powders is a comparatively easier problem than that of orange juice powder on account of the much wider safety margin in their case (4, 10 and 14% RH as against 1% RH). It may, however, be possible to increase the safe margin for orange juice powder also by reducing its initial moisture content to a slight degree.

In the case of all the products except mango custard powder, there was visible cake formation even in the range of 2.3–2.8% moisture level. The high moisture level of 4.4% for caking in the case of mango custard powder may be attributed to additives like starch and skim milk powder. There was mould growth when the RH was greater than 80 to 85%. It is to be seen to what extent these storage characters will be affected by packing the products in containers like polyethylene bags, etc. Work on this aspect of the problem is in progress.

SUMMARY

The data for fruit powders and fruit custard powder show that the graphical interpolation method, which is much quicker than the weight equilibrium method, can be employed for the quick and approximate determination of their ERH. The latter method, however, gives more precise data and also detailed information regarding the storage behaviour of the product in relation to danger and critical points and safe margin, especially when products undergo visible changes in colour, texture, etc.

Mango and guava powders have almost similar moisture equilibrium curves, while orange juice and mango custard powders have curves that differ significantly from these and also from each other. The fruit juice powders and custard powder vary widely as regards the range of ERH in relation to uptake of moisture, mango and guava powders passing through a wider span of ERH than orange juice and mango custard powders.

Mango and guava powders and mango custard powder have a wider safety margin (4, 10, and 14%

Table 5

Initial, critical and danger points for moisture content and relative humidity for fruit powders (at 37° C)

Product	Initial point (I)		Danger point (D)		Critical point (C)		Remarks	
	Moisture %	RH %	Moisture %	RH %	Moisture %	RH %	Moisture content of product	Condition of the product
Mango Custard Powder. I.	2.0	13.0	4.2	27.0	4.4	32.0	4.4	Free flowing. Colour not affected.
							4.7	Cake formation. Colour dull.
							7.3	Cake formation. Product brownish in colour.
							9.6	Cake formation. Product deep brown in colour.
							18.0	Mould growth.
Orange Juice Powder. II.	1.9	39.0	2.0	40.0	2.8	45.0	2.8	Cake formation.
							4.6	Caking. Product dark brown in colour.
							7.3	Caking. Product deep dark brown in colour.
							20.0	Mould appeared.
Guava Powder. III.	1.1	47.0	1.7	57.0	2.3	62.0	2.3	Cake formation. Colour normal.
							2.5	Cake formation. Product brown in colour.
							8.8	Mould growth.
Mango Powder. IV.	1.4	58.0	1.8	62.0	2.5	67.0	2.5	Cake formation.
							4.1	Mould growth.
Banana Powder. V.[1]								

[1] Complete data not available.

RH) than orange juice powder (1% RH) and therefore present a less critical packaging problem. All the products have a tendency to develop mould growth, when the RH of the storage atmosphere exceeds about 80%.

Lumping and caking were observed in the range of 2.3–2.8% moisture level in the case of guava, mango, and orange juice powders and at 4.4% level in the case of mango custard powder. This increase in moisture level in the case of mango custard powder may be attributed to the additives like skim milk powder and starch.

Acknowledgment

The authors wish to express their gratitude to Dr. V. Subrahmanyan, Director, and Dr. Girdhari Lal, Assistant Director, for their keen interest in this investigation.

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Notes and Letters

Objectives of the QM Food and Container Institute for the Armed Forces Radiation Research Program[a]

This symposium[b] is primarily concerned with the microbiological aspects of food irradiation. There is no need to tell this group that it is one thing to apply ionizing radiations to inert matter and quite another to turn its invisible powers against living organisms. In the latter instance, we must assume a bio-physico-chemical structure that is able to compensate for disturbing effects.

The Radiation Preservation of Food Program is now in its seventh year. Fundamental objectives of the broad program are, essentially, the same today as when formulated early in the program. In leading up to the microbiological aspects of food irradiation, it seems worthwhile to dwell briefly on the objectives of the whole program. A number of these objectives are reflected in our microbiological objectives. Here then are the over-all objectives in food irradiation.

1. To investigate the possibilities of preserving foods by ionizing radiation.

2. To establish a reservoir of basic scientific information for future improvement of radiation processed foods.

3. To advance the technology of food radiation processing and to better understand the fundamentals involved.

[a] Approved for release by the Department of Defense and registered as No. 2011 in the series of articles reporting the work of the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command.

[b] Presented at the Symposium on the Radiation Preservation of Food, Twentieth Annual Meeting of the Institute of Food Technologists, May 18, 1960, San Francisco, California.

4. To investigate the possibilities of furnishing ration components, sterilized by ionizing radiation, to troops in the field.

5. The establishment of the wholesomeness of irradiated food.

The possibility of sterilizing foods "in the cold" offered—and still does—a challenge to those interested in food preservation. "Cold" sterilization offers impressive advantages. If microorganisms can be killed by ionizing radiation and spoilage prevented, then an improvement in quality, flavor and texture can be realized in items that ordinarily suffer deterioration through other means of processing. Preliminary experimentation showed the feasibility of preserving foods by ionizing radiation. These same experiments, however, pointed out the obstinate fact that radiation is detrimental to the quality of some foods. The nature of this detrimental effect was well enough defined to indicate specific lines of research. It was possible to articulate a set of research tasks directed toward the basic factors involved in such other deficiencies. Basic research was started on the changes in flavor, color and texture of foods treated with ionizing radiation, the effect on food enzymes, the wholesomeness of foods so treated, the measurement of radiation dose, and, in the area of our interest at this meeting, basic and practicable research in the field of microbiology.

OBJECTIVES OF THE MICROBIOLOGICAL SECTION OF THE PROGRAM

Microbiology extends into a broad area but essentially we are dealing, with respect to radiation, with three specifics:

1. With the interrelationships of radiation dose and the killing of microorganisms.
2. With the condition and quality of raw food products treated.
3. With the actual mechanisms of the killing of the microorganisms involved.

I shall now endeavor to point out the objectives of the microbiology section of the radiation preservation of food program in its interrelated form.

One of the first objectives is to determine the ways and means of destroying microorganisms that cause food spoilage and/or those of public health significance. Accomplishment of such an objective is not as easy as it sounds, for as in other means of food preservation, the destruction of microorganisms in foods is dependent upon the influence of the substrate, temperature, pH, quality and type of food, etc., as well as the species, numbers and condition of the microorganisms themselves. You will agree, I am sure, on the complexity of these problems and the difficulty of demonstrating the precise influence of any one of these factors, let alone their interrelationships.

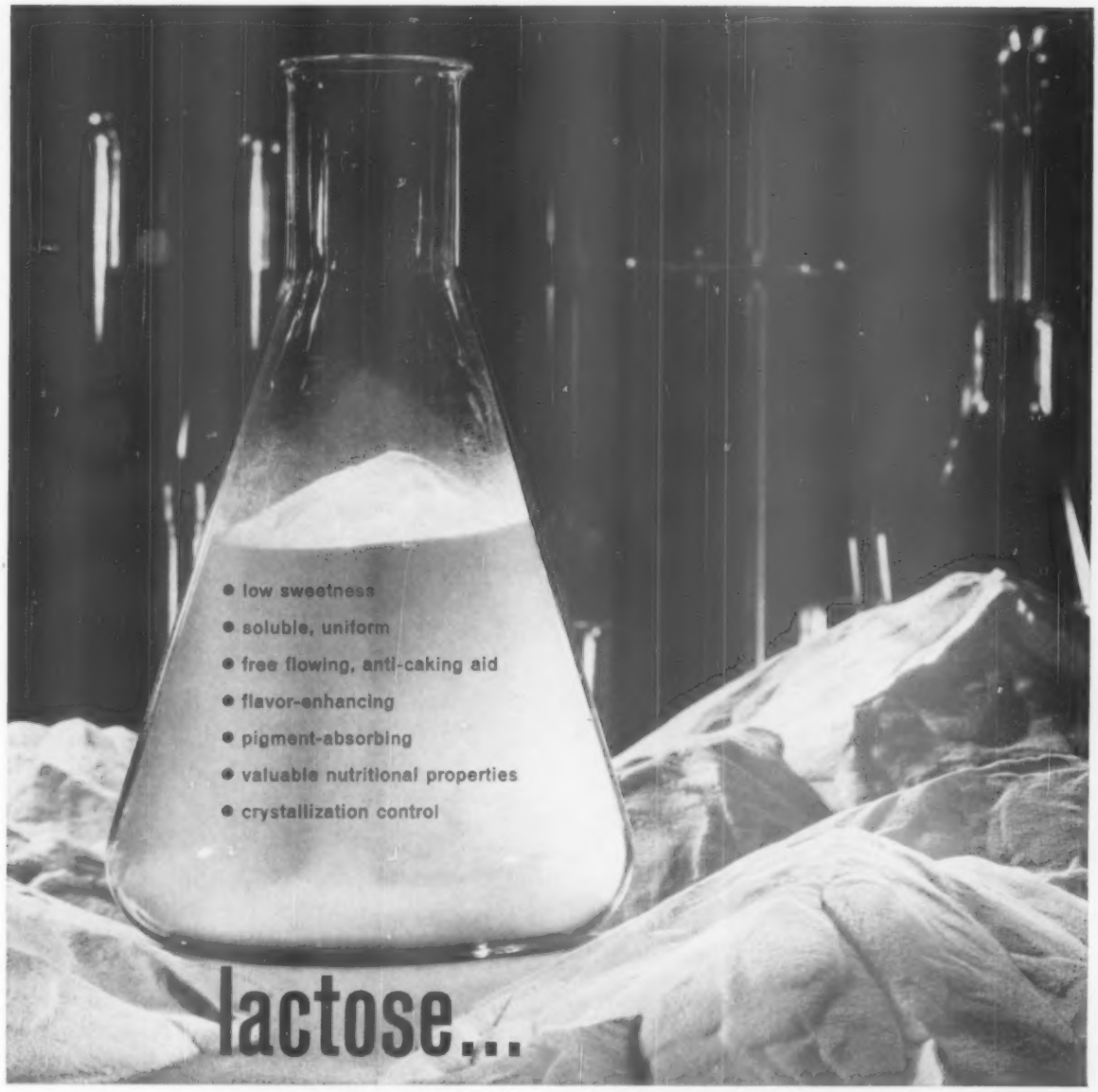
Therefore, before this objective can be gained many facets of the basic research involved must be explored. It will be necessary to study the physiology of the microorganisms of interest to determine their sensitivities to ionizing radiation under a wide variety of circumstances. The effect of radiation on the biochemistry of bacterial cells and of spores must be explored. Although there are answers in certain areas regarding cells and spores, the introduction of the radiation variable requires that additional studies be made and in a new light.

In the processing of food by thermal energy, it is found that several of the organisms responsible for food spoilage are more resistant to heat than is *Clostridium botulinum*. In processing food by ionizing radiation, the reverse is true. Since *Clostridium botulinum* is of profound public health significance, it follows that the destruction of this organism is of primary concern. Permit me to emphasize that point. Research on the processing of food by radiation must be based on the problems presented by the organisms of this species. Specifically, studies must be, and will be, undertaken to establish the radio-resistance of the various types and strains of *Cl. botulinum*—this in order to establish a definitive sterility reference for processing by ionizing energy. In order to complete these studies, it will be necessary to observe cultures at all stages of development, the nutrition and physiology of cell growth, spore formation, and spore germination.

Some of these factors may hold the key to more effective killing of the organisms and the consequent establishment of lower radiation doses.

Radiation dose level. This leads to another of the objectives of the program, the establishment of a radiation dose level at which the safety of food products is assured and also their quality. Thermal processing times and temperatures are based upon long experience in the canning industry. Basic information has been accumulated through many researches involving the processing of millions of cans of product, making it possible to calculate processes and to establish the "cook" necessary to establish safety and keeping quality. Since almost no information has been available in the area of preservation by radiation, no such precise values can be established. It is true that some work on the mathematics of radiation sterilization has been done but, by and large, it has been necessary to draw upon the knowledge available in the canning industry to shape up a concept of safety. Thus, a value of 4.46 megarads of dose has been calculated, using the 12D concept of the canning industry, as the level necessary to establish safety in non-acid food products. To many workers in the field, this dose seems too high. However, at the present time, we simply do not have the data on which to base a recommendation for reduction.

It will be necessary to devise new techniques and experimental designs specifically calculated to obtain radiation data comparable to those available for thermal processing. Such new techniques would involve the employment of methods which alter the susceptibility of the microorganisms to the radiation. The hope is to find ways and means of changing the environment of microorganisms from a good one to a bad one. Such methods, as changes in pH, electrolyte content, moisture, soluble solids, and pretreatment of raw materials, must be thoroughly investigated; as



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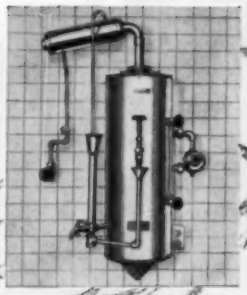

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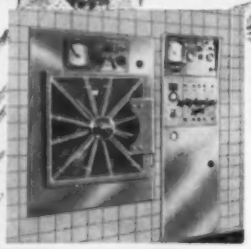

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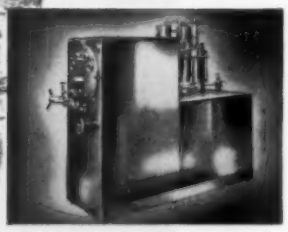

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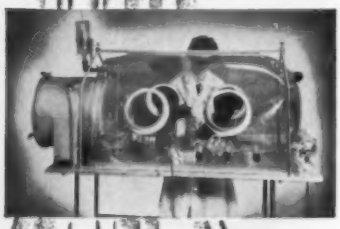

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well as employment of combination treatments, such as heat, antibiotics, germicides, bacterial inhibitors or activators of spore germination. These procedures offer considerable promise of assisting in lowering the necessary radiation dose. Some work along these lines has been accomplished or is presently underway, but much is yet to be done.

If the radiation dose necessary for safety is found to be less than 4.46 megarads, a bonus of better quality-processed foods can be realized. High doses of radiation bring about quality deterioration, especially in color, flavor and texture; such deterioration is less at lower doses. Many workers have found no spoilage or toxin production at radiation doses below 4.46 megarads but it remains for carefully planned research to establish scientifically, the killing dose necessary for safety from the possible toxin production by *Cl. botulinum*.

This naturally leads to the next objective: devising dosimetry methods to confirm or validate the established dose. In other words, reliable methods assure reliable answers. When the program started, there were no methods available for measuring high doses of radiation accurately. It was necessary to develop dosimeters and dose measurement techniques before reliable dose measurements could be made. There was no assurance that a programmed dose was actually being received. Accuracy of dose measurement is essential to radiation research on the killing of microorganisms. The accurate measurement of absorbed radiation dose is just as essential as the measurement of heat absorption in thermal processing. Chemical dosimetry methods are available for measurements but they are time-consuming, costly, and the results cannot be read continuously or in the very short time intervals permitted when radiation processing is underway.

Constant monitoring required. It will be necessary to develop a means of constantly monitoring radiation doses, especially when an electron accelerator is used, so that a container that has not received the full required dose throughout its contents may be identified. Gamma fields that are uniform in dose distribution need only be monitored at intervals, but electron beams will have to be monitored at microsecond intervals due to the way the dose is delivered. You can readily see that dosimetry is an intriguing field of study. Until there are rapid and accurate methods of determining the radiation dose absorbed, there cannot be assurance of the safety of the food pack. The assurances of heat sterilization are pretty well worked out. The assurances in the field of radiation sterilization are in the early stages, but we are making headway in the development of radiation measuring instruments comparable to the thermometer.

This leads us to the next objective: to develop fundamental information on the biological effect of various types of radiation sources and the manner in which such doses are delivered. From a theoretical standpoint, the absorption of identical amounts of radiation energy from each type of source should produce the same biological effect. Also, the delivery of the total dose in increments should not alter the biological effect. However, there have been reports that differences in biological effects have been noted. These have been sufficiently frequent to make it necessary to resolve this point. Research must be done under a very carefully planned program to determine whether in truth there are differences due to type of source, manner of delivering the energy, or in delivering a fractionated dose. These results will have to be weighed against the possibilities that such reports have resulted from insufficiently considered differences in experimental conditions, accuracy of dose measurement, effect of substrate, difference in time intervals and other factors which were independent of the energy being absorbed and which may have given rise to such anomolies. Once again, may I reiterate that we are in an area where we are dealing with live organisms—and that means that any form of energy put into these organisms, any change in the environment, any disturbance whatsoever must be studied for its significance.

The establishment of microbiological safety of irradiated foods is an objective that cannot be overlooked. Therefore, the development of test procedures to establish safety must be made. The mere designation of processing requirements believed to render the product safe and commercially sterile is not sufficient. In the food processing industry, several procedures are in use to detect potential spoilage of a pack. Since *Cl. botulinum* survives radiation doses lethal to most spoilage organisms, conventional methods will not apply and a new type of testing will have to be devised. This could involve the use of samples of the product inoculated with a nonpathogenic microorganism of a higher radioresistance than *Cl. botulinum* or a very rapid, accurate and inexpensive method for detection of botulinum toxin. The development of such methods would permit monitoring the processing of food through a statistical sampling procedure.

Enzyme-inactivation. Enzymes play important roles in food preservation. This brings up another objective—to better understand the enzyme systems of the plant and animal food materials, and the enzyme systems of the microorganisms present in order to control quality deterioration. Most enzymes are more radio-resistant than tissue systems, and therefore may not be inactivated by the ionizing radiation treatment. We know that residual enzymes can cause food deterioration. In cases where the food is cooked or properly enzyme-inactivated, this is not a problem; but in unblanched products or fresh products, irradiated to reduce spoilage, it could be a problem. It is entirely possible that a treatment to block or inhibit the enzyme systems of bacteria may be found. Such a breakthrough could render the bacteria incapable of multiplying and causing damage. Also, such treatment could block the activity of residual enzymes in killed cells. It is not unreasonable to expect that within the frame of safety and wholesomeness, a lethal disturbance that will upset the mechanism of the enzyme systems of bacteria can be found.

Packaging of irradiated foods. We come now to packaging. The objective here is to develop a satisfactory flexible packaging material that will prevent recontamination of food material even under conditions of rough handling and long-term storage. Certain packaging materials show marked promise for irradiated foods but possess certain limitations. In addition to the effect of radiation on the packaging material, any creasing, folding, scratching or breaks caused by rough handling render many films unsatisfactory from a bacteriological point of view. It becomes necessary in fact to laminate two or more films to assure that a package can be made that is impervious to micreorganisms. From a logistic point of view, flexible containers meet many needs such as being lightweight, packing and carrying easily, having minimum residual material after contents are used, lower over-all cost, etc. There is need to find tougher films that will withstand rough handling at all stages in the supply line and still prevent the re-entry of microorganisms into a sterile package.

Although the main interest of the Military lies in using ionizing radiation to accomplish sterility of foods and thus providing a product that can be shipped overseas and stored without refrigeration, there is also, for logistic reasons, an interest in low dose radiation of certain foods. Low dose radiation of these foods would permit delivery of fresh materials to overseas bases in better condition than by another means. This too is an objective—although at this time of lesser interest than the field of sterile foods.

THE PRESENT OUTLOOK

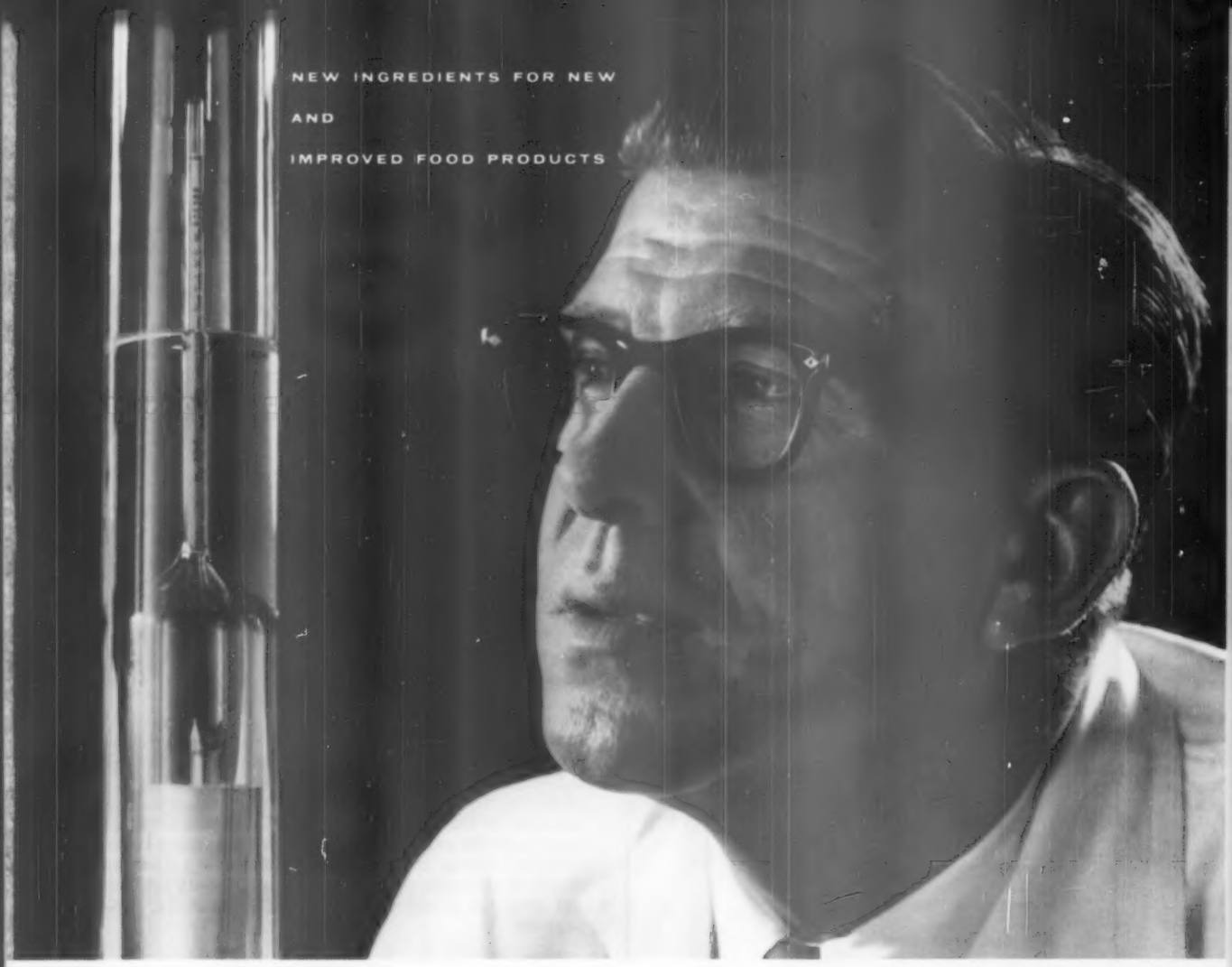
Certain raw products can be shipped without refrigeration today to troops in overseas bases or in the field. However, some deterioration takes place, especially that effected by rotting and molding. If these deteriorative agents can be markedly reduced by low-level radiation, much waste can be prevented and the process will serve a genuine purpose. It is disappointing, to say the least, to spend shipping space and weight on food that is never eaten. A means of extending the shelf or shipping life of these products could provide a means of getting nutritionally valuable foods into areas where they cannot be sent at present. This would be particularly valuable in overseas regions where, in peacetime, some refrigeration is available. The interest in this phase of the program is keen but there is need for research to determine the precise extent to which the shelf life of fresh items can be extended by this means for the benefit of the Armed Forces. In other words, none of our research is done outside the common sense reasoning of the logisticians.

Several objectives of the Radiation Preservation of Food Research Program have been outlined and discussed with you. The Military Program does not presently embrace all of these areas nor is it contemplated that work will be undertaken in all of them in the near future. It has been my purpose to point out those areas in which there are definite problems to be solved—problems that can be expected, with considerable assurance, to yield to this new processing method. As time goes on and some of the problems are solved, it will be desirable to move in on the lesser problems.

We have developed in the few years that we have been at work on radiation preservation, some ingenious methods of attack and the solution to some problems. We are building up a reservoir of basic information that we can eventually tap for use in an ionizing radiation process. More data are needed in the reservoir to be assured of a steady and powerful flow. Our objectives, as I trust have been shown, are designed to bring in more basic data and to eventually lead to the solution of some of the food processing problems involved in the use of ionizing radiation.

HARRY E. GORESLINE
Deputy Scientific Director
QM Food and Container Institute for the Armed Forces,
Chicago, Illinois

May 10, 1960



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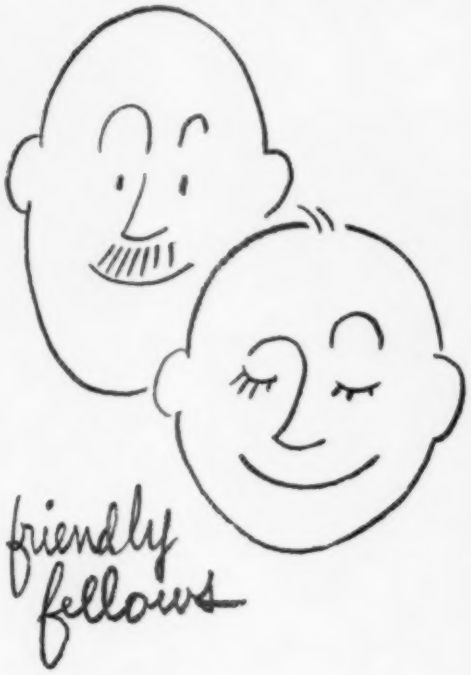

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What more is there to say?

Food additives *Food container components*

Problems relating to the extractability of food container components. L. E. Schniepp, H. O. Etian, and J. M. Jackson.

Under present circumstances, it is possible to say that the organic chemical ingredients now in use in the manufacture of food containers either do not contribute additives or the use is in compliance with established regulations. It is believed that the container industry has a sufficiently broad background of experience with various materials and test procedures to handle the problems relating to new materials and to assure continued compliance with the Food Additives Amendment of 1958. (See page 496).

Food additives *Biological screening techniques*

Biological screening techniques for food additives. Margaret Ives and Edythe M. Nagler.

The biological screening method for toxicity described here is useful for eliminating from consideration as food additives materials which are grossly toxic. A material which appears toxic in this test will be toxic at lower levels in a chronic two-year test. It does not follow, however, that a material which is non-toxic in a 90-day subacute test will be non-toxic on a two-year feeding study. If the material passes this screening test and still shows promise from the technological standpoint, a two-year test will be required to obtain information for a petition under the Food Law Amendment. In the tests used as examples in this paper, only materials of unknown biological activity were considered. Before embarking on any biological testing, it is always advisable to do a literature search to determine what information is already available and plan the experiment from that information. (See page 499).

Food additives *Food colors*

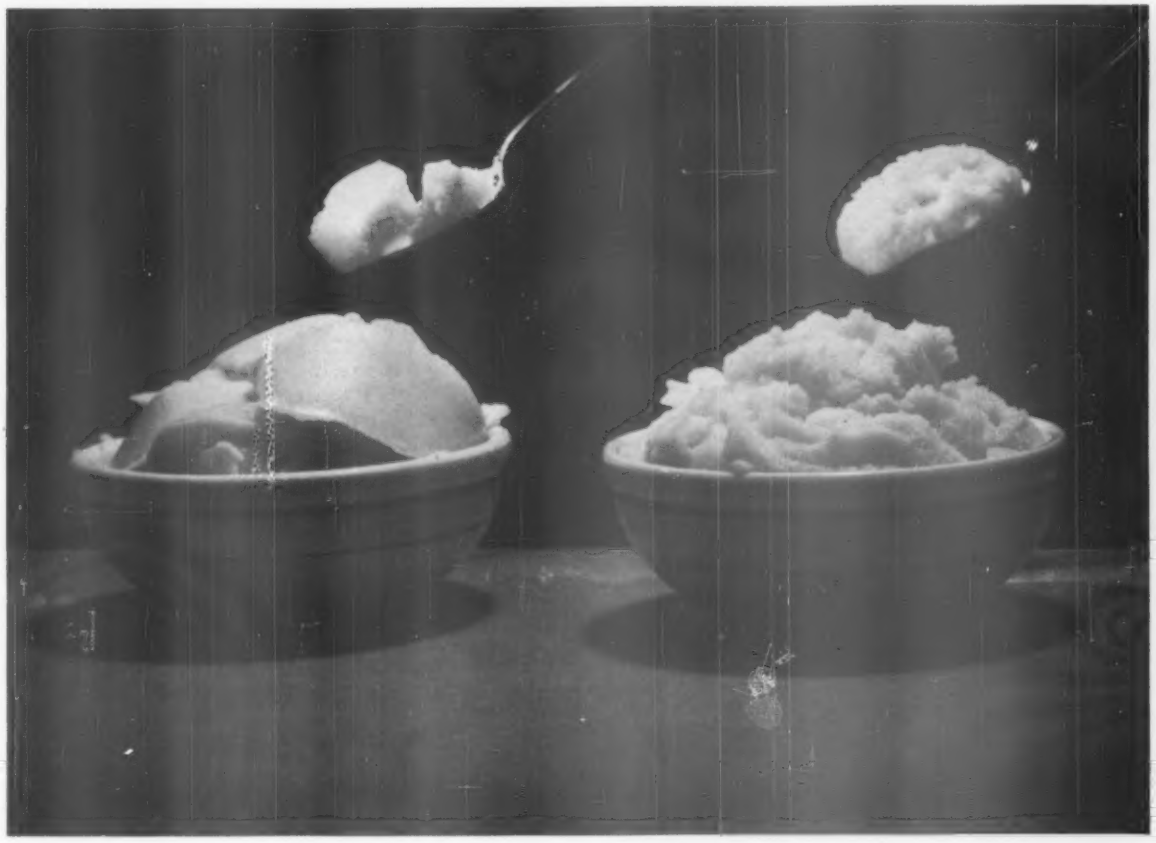
Food colors—their status under the law. Arthur T. Schramm.

The viewpoint of the color industry with regard to the so-called Delaney cancer clause of H. R. 7624 [Section 706 (b) (5) (B)]. S 2197 is also discussed. The color industry would prefer to leave it to the Secretary of the Department of Health, Education, and Welfare to deal with carcinogenic manifestations but would not object to a provision giving the Secretary discretionary rather than mandatory authority. (See page 503).

Food additives *Antibiotic*

Antibiotic preservation of beef with subsequent feeding to experimental animals. R. B. Sleeth, J. C. Armstrong, H. S. Goldberg, and H. D. Naumann.

Raw and cooked meat containing low levels of OTC, when fed over a period of 16 weeks, did not cause any detectable residual in blood serum, heart, kidney or muscle of rats. Apparently, no significant changes occurred in the gut flora of rats following low-level exposure to OTC for 16 weeks. On the basis of this study, the potential health hazard of the consumption of meat containing low levels of OTC would appear to be small. (See page 505).



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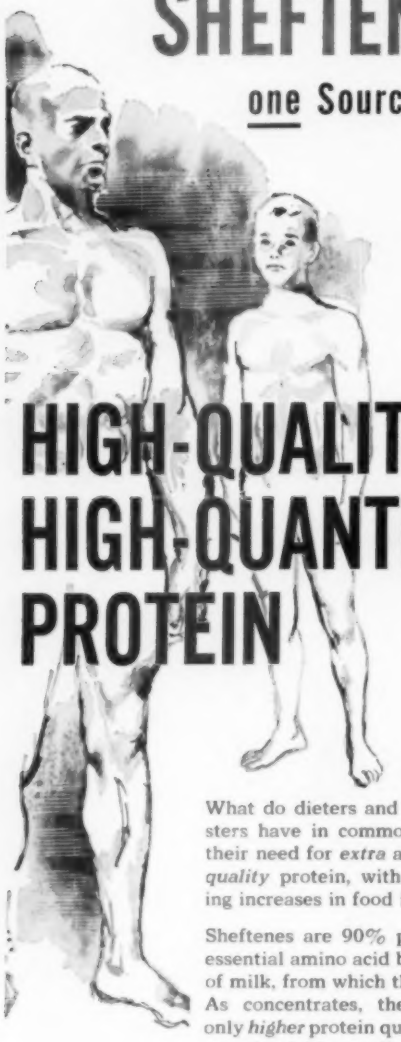

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Review — *Nutritional, chemical changes in heated fats*

Nutritional and chemical changes occurring in heated fats: a review. Edward G. Perkins.

Practical research on the formation and accumulation of polymeric materials in oils obtained from deep frying operations has not received enough emphasis in published work. Literature concerning chemical determination of polymeric materials in fatty acids obtained from commercial operations is not readily available. Differences in the temperatures of heating and oxygen content can alter the rate and types of polymerization. The length of time that an oil is exposed to oxygen and heat, and especially the percentage of heated oil in the diet, seem to have as great a bearing on the nutritive value of the oil as temperature of heating. It can be observed that most of the data derived from this type of research cannot be projected to predict whether an unsaturated oil will be damaged sufficiently during processing and commercial frying operations to be harmful. The data obtained is sufficient, however, to justify the suspicion that the use of such oils containing polymeric materials may not be desirable from a nutritional point of view. It is obvious that practical research must yet be done in order to answer at least three main questions: a) Are polymers formed in unsaturated oils during deodorization, processing, and use? b) Are polymeric materials absorbed on food products and if so to what extent? c) What are the physiological and nutritional effects of these materials? (See page 508).

Consumer opinion — *Sweeteners in citrus drinks*

Consumer opinion of sweeteners in frozen concentrated lemonade and orange juice drink. Rose Marie Pangborn, George L. Marsh, W. R. Channell, and Horace Campbell.

Three surveys were conducted among 320 families to determine consumer opinion of amount and type of sweetener in frozen, concentrated lemonade and in frozen, concentrated orangeade: Study I—Determination of optimum sweetness among 4 sucrose-sweetened concentrates, Study II—Effect on acceptance of dextrose replacement of 10, 20, and 30% of the sucrose, Study III—Effect on acceptance of corn syrup replacement of 10, 20, and 30% of the sucrose. In Study I, an optimum sweetness level of 11° Brix at a Brix-acid ratio of 16.0 was established for lemonade whereas 13° Brix at a Brix-acid ratio of 18.5 was optimum for the orangeade. Only the lemonade sample containing 30% dextrose replacement was rejected by the consumers; all orangeade containing dextrose was acceptable. However, undesirable darkening was observed in all dextrose-containing samples after 6 months of storage at 0° F. Corn syrup was objectionable in lemonade, but inconclusive results were obtained for orangeade. The laboratory taste panel found that 9.8° Brix in the all-sucrose sweetened lemonade was equivalent in sweetness to 10.8° Brix in samples with sucrose replacements of 20% dextrose or 20% corn syrup. Sucrose had the greatest sweetening capacity and corn syrup had the least. (See page 515).

(Continued on page 15 following Technical Section)

(Continued from page 12 preceding Technical Section)

Antibiotic and radiation *Applied to poultry*

Experimental preservation of poultry by chlortetracycline and radiation. A. W. Phillips, H. R. Newcomb, and F. C. Bach.

Ten ppm of chlortetracycline and 100,000 rads of ionizing radiation were used in an experimental antibiotic-radiation process for retarding microbial spoilage in prepackaged fresh chicken meat. Treated meat was stored at 41° F for 7 weeks without bacterial spoilage. Results obtained on meat independently treated with either radiation or antibiotic were inferior to those achieved with the combination process. (See page 521).

Frozen foods technology *Frozen spinach*

Time-temperature tolerance of frozen foods. XXIII. Quality changes in frozen spinach. W. C. Dietrich, Mildred M. Boggs, Marvel-Dare Nutting, and N. E. Weinstein.

Time-temperature conditions ranging from one day at 40° to two years at −20° F have been investigated for spinach with regard to changes in chlorophyll, ascorbic acid, carotene, reflectance color, visual color, and flavor. Significant deterioration did occur (except for carotene) above 0° F within periods of handling that may be experienced in industry practice. Measurable rates increased by a multiple of 10 for about each 15° rise in temperature between 0° and 25° F. This increase in rates was similar to the temperature effects on rates found earlier for peas and green beans. Comparison of change-in-percentage chlorophyll converted to pheophytin in spinach with the change in peas and green beans, for example at 10° and 20° F, showed conversion in spinach to increase 1 to 2 times as fast as in peas and one-half to one-quarter as fast as in green beans. Loss of ascorbic acid in spinach (expressed as mg/100g) was 1 to 2 times as much as found in green beans and peas under the same conditions. Some time-temperature effects caused enough deterioration for industry panels to rate samples as "sufficiently poor to cause consumer complaint." Data emphasize a large variation among 10 oz packages of spinach and the necessity for adequate sampling for quality evaluation. (See page 522).

Oxidation method *For estimating storage life of frozen seafoods*

An accelerated oxidation method for the estimation of the storage life of frozen seafoods. Robert E. Palmateer, T. C. Yu, and Russell O. Sinnhuber.

An accelerated autoxidation procedure for frozen seafoods and fats and oils is presented. This method depends on the use of purified diatomaceous silica (Celite) as a support for the material to be tested. The great increase in surface area that results permits autoxidation to proceed at an accelerated rate even at sub-freezing temperatures. The effect of temperature on the rate of autoxidation was determined as well as the effect of fluctuating temperatures on the storage of frozen seafoods. The application of this procedure to evaluate antioxidants is demonstrated. The proposed method is useful for the

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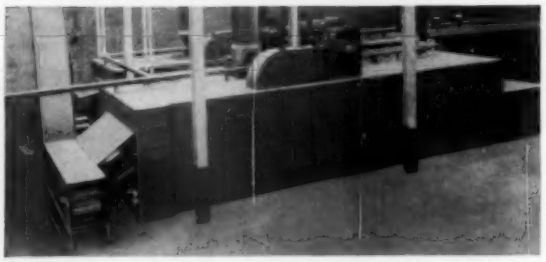

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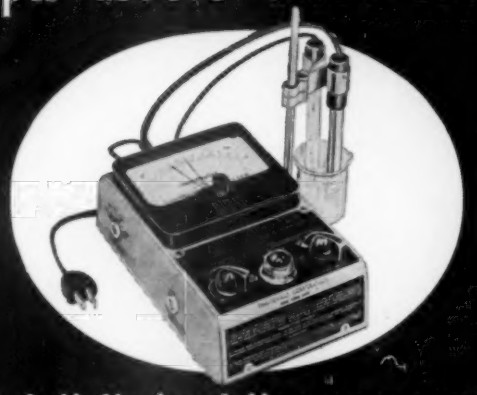

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accelerated oxidation of fats and oils. The effect of the antioxidants, quercetin and propyl gallate on the stability of a lard substrate is shown by this method. The surfactant, Celite, does not interfere with the peroxide or TBA methods for the determination of oxidative rancidity. The possible use of this accelerated autoxidation method for the accelerated storage of frozen vegetables and other foods is suggested. (See page 528).

Equilibrium RH relationships — *Fruit juice powders*

Equilibrium relative humidity (ERH) relationships of fruit juice and custard powders. G. S. Siddappa and A. M. Manjundaswamy.

The data for fruit powders and fruit custard powder show that the graphical interpolation method, which is much quicker than the weight equilibrium method, can be employed for the quick and approximate determination of their ERH. The latter method, however, gives more precise data and also detailed information regarding the storage behaviour of the product in relation to danger and critical points and safe margin, especially when products undergo visible changes in colour, texture, etc. Mango and guava powders have almost similar moisture equilibrium curves, while orange juice and mango custard powders have curves that differ significantly from these and also from each other. The fruit juice powders and custard powder vary widely as regards the range of ERH in relation to uptake of moisture, mango and guava powders passing through a wider span of ERH than orange juice and mango custard powders. Mango and guava powders and mango custard powder have a wider safety margin (4, 10, and 14% RH) than orange juice powder (1% RH) and therefore present a less critical packaging problem. All the products have a tendency to develop mould growth, when the RH of the storage atmosphere exceeds about 80%. Lumping and caking were observed in the range of 2.3–2.8% moisture level in the case of guava, mango, and orange juice powders and at 4.4% level in the case of mango custard powder. This increase in moisture level in the case of mango custard powder may be attributed to additives like skim milk powder and starch. (See page 533).

Radiation preservation — *The Quartermaster program*

Objectives of the QM Food and Container Institute for the Armed Forces radiation research program. Harry E. Goresline.

The Radiation Preservation of Food Program is now in its seventh year. Fundamental objectives of the broad program are, essentially, the same today as when formulated early in the program. The over-all objectives in food irradiation are: 1. To investigate the possibilities of preserving foods by ionizing radiation; 2. To establish a reservoir of basic scientific information for future improvement of radiation processed foods; 3. To advance the technology of food radiation processing and to better understand the fundamentals involved; 4. To investigate the possibilities of furnishing ration components, sterilized by ionizing radiation, to troops in the field; 5. The establishment of the wholesomeness of irradiated food. (See page 537).

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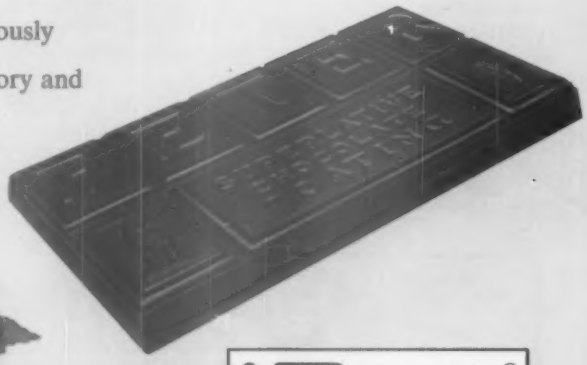

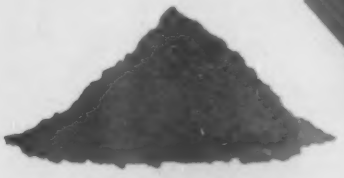

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1961 Technical Program

Plans for the 1961 Technical Program are moving ahead.

Papers can be sent to any Committee member (See September issue) but are best sen to the Chairman of that Division in which the discipline is listed. The deadline for titles is December 1, 1960. Abstracts of all papers must be in the hands of the Session or Division Chairman by January 1, 1961. Abstract forms will be provided.

The selection of all subjects has not been completed, but the following can now be announced:

Flavors and Colors — Session Leader: Dr. Arthur S. Wendt, Fred Fear and Company, Foot of Joralemon Street, Brooklyn 1, New York.

Pressurized Foods — Session Leader: Dr. John M. Jackson, American Can Company, Research and Development Center, Barrington, Illinois.

Food Additives—Session Leader: Dr. John F. Mahoney, Merck and Company, Rahway, New Jersey.

An Educational Symposium entitled: *Food Technologists, Undergraduate or Graduate* — Session Leader: Dr. Roy E. Morse, Thomas J. Lipton, Inc., Hoboken, New Jersey.

Statistical Quality Control—Session Leader: Dr. Bruno A. Filice, Filice & Perrelli Company, 1200 South Tenth Street, Richmond, California.

Quality Control — Instrumentation — Session Leader: Mr. James B. Anderson, H. J. Heinz Company, 11 Rosemont Avenue, Pittsburgh, Pennsylvania.

FRED C. BASELT, Chairman
PAUL A. BUCK, Vice Chairman

DIXIE

The Dixie Section of IFT held its Summer Meeting on August 12, 1960 at the Dromedary Division of National Biscuit Co. at Woodbury, Ga. The group met at 10:00 a.m. for a tour of their Pimiento Cannery and Date Packing Plant. From Woodbury the group went to Pine Mountain Tavern in Roosevelt State Park for lunch and the program.

Mr. Ralph Bohn, chairman, called the meeting to order. Mr. Charles Lovvorn introduced the speaker, Mr. Jack R. Chappel, Officer-In-Charge, Processed Products Inspection Service, Fruit and Vegetable Division, Agricultural Marketing Service. Mr. Chappel gave some interesting facts about the inspection service and how it works. He also showed models used by the inspectors to establish the quality of the various processed fruits and vegetable products which are packed under the USDA program.

Several members and their families went by Callaway Gardens and Beach for a swim before journeying home.

FUTURE MEETINGS FOR FOOD TECHNOLOGISTS

1960	
October 12-14	The Seventh National Symposium of the American Vacuum Society, Cleveland-Sheraton Hotel, Cleveland, Ohio
November 12-19	Fifth Pan-American Congress of Pharmacy and Biochemistry, Santiago, Chile. (For information address Senor José Pérez Molina, Secretary, Box 6084, Santiago, Chile).
1961	
May 15-18	Fourth International Congress on Canned Foods, Berlin, Germany (for information address M. Henri Cheftel, J. J. Carnaud & Forges de Basse Indre, 71 Avenue, Edouard Billancourt, Seine, France).

1961	
May 7-11	**Twenty-first Annual Meeting of the Institute of Food Technologists, Hotel Statler, New York, New York.**
1962	
June 10-14	**Twenty-second Annual Meeting of the Institute of Food Technologists, Fontainebleau Hotel, Eden Roc Hotel, Miami Beach, Florida.**
1963	
May 19-23	**Twenty-third Annual Meeting of the Institute of Food Technologists, Pantlind Hotel, Civic Auditorium, Grand Rapids, Michigan.**

An open invitation is extended to readers of FOOD TECHNOLOGY to send in to the Editorial Office, 11606 South Bell Avenue, Chicago 43, Illinois notices of annual or national meetings of interest to food technologists.

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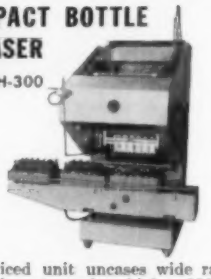

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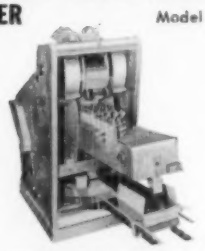

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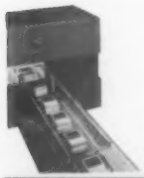

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ABSTRACTS

General Foods Research Center
Selected by H. A. Campbell

ANALYTICAL METHODS

Programmed temperature gas chromatography.

DAL NOGARE, S. AND LANGLOIS, W. E. (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). *Anal. Chem. 32*, 767–70 (1960).

A derivation of solute retention behavior in programmed temp. gas chromatography is presented. The final equation, for the dependence of retention time on heating rate, mass flow rate of carrier gas, and starting temp., was integrated by computer for a linear temp. program, and yielded results in good agreement with expt. A correlation is noted between relative retention time and the ratio of flow rate to heating rate. Under identical linear temp. programming conditions, increasing the liquid phase polarity increases the retention time of each member of a polar homologous series by the same amt.

Molecular sieves as subtractors in gas chromatographic analysis. III. The secondary effect of the molecular sieve trap column.

ETTRE, L. S. AND BRENNER, N. (Perkin-Elmer Corp., Norwalk, Conn.). *J. Chromatography 3*, 235–8 (1960).

Using molecular sieves as trap column for specific adsorption of individual components from mixts., a secondary effect was observed in the case of org. compds. contg. carbonyl or hydroxyl groups. As a result of this secondary reaction, the substance which would pass the column is retarded under the influence of the adsorbed component. This effect might probably be attributed to an aldol condensation or formation of secondary hydrogen bonds. These reactions could promote the basic character of the molecular sieves. In the case of hydrocarbons, no secondary adsorption effect was noted.

A preparative column for the ion exchange isolation of amino compounds.

TALLEY, E. A. AND PORTER, W. L. (Eastern Regional Research Lab., Philadelphia, Pa.). *J. Chromatography 3*, 434–7 (1960).

Since analytical ion-exchange methods are micro in scope, other more involved and more tedious procedures must be used to isolate unknown materials for identification. This paper reports the results of scaling up the Moore and Stein (*J. Biol. Chem. 219*, p. 623) 110 cm. column to the extent of 24 times. By the use of this column, sufficient materials for analysis and prepn. of derivs. can be obtained with a minimum of effort and change in the ordinary analytical method.

A method for the quantitative separation of fatty acids from unsaponifiable matter.

SAMMONS, H. G. AND WIGGS, S. M. *Analyst 85*, 417–18 (1960).

Fatty acids have been sepd. from unsaponifiable matter by adsorption on alumina from soln. in light petroleum. The acids are quantitatively removed by conversion *in situ* to their methyl esters followed by elution with diethyl ether. The recovery of both unsaponifiable matter and fatty acids, as methyl esters, is greater than 90%.

A study of atomic absorption spectroscopy.

MENZIES, A. C. (Hilger & Watts Ltd., London, Engl.). *Anal. Chem. 32*, 898–904 (1960).

Flame photometry has been used for many years to analyze materials which may be brought into soln. For certain metals, notably the alkali and alkaline earth metals, the method has become standard. However, its scope is restricted and not until 1955 did Walsh suggest analyzing by flame absorption instead of emission. The newer technique is called atomic absorption spectroscopy. The technique and some of the difficulties in its application, as well as some examples of its use, are presented.

An examination of the occurrence of honeydew in honey.

KIRKWOOD, K. C., MITCHELL, T. J. AND SMITH, D. (Royal Coll. of Sci. & Tech., Glasgow, Scotland). *Analyst 85*, 412–16 (1960).

Honey contg. honeydew, the sweet and sticky excretion of certain leaf-sucking insects, is not a safe food for bees; a simple means of distinguishing honeydew from floral honey is described. Forty-two honeys were analyzed for moisture, colloid, nitrogen, dextrin, reducing sugars, free acidity and ash, and values for specific rotation and pH were measured. The exptl. results were subjected to discriminatory analysis, and a linear discriminant function was evolved, based on the results for ash, pH and reducing sugars; the numerical value of this function for any sample serves to classify the sample as floral or honeydew in origin.

BIOLOGICAL SCIENCES

Biochemistry

Studies on a protein-synthesis-affecting substance from biological materials.

HEADEC, J. AND STROUFOVA, A. (Oncological Inst., Prague, Czechoslovakia).

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Biochim. et Biophys. Acta 40, 32–42 (1960).

A substance of lipid character contg. carbohydrate and phosphorus which stimulates the incorporation of labelled amino acids into the protein of Ehrlich ascites cells was found in animal tissues and egg yolk. The content of the tissues of tumor-bearing individuals was higher than that of the tissues of normal ones. The substance was obtained in a crystalline form.

Irradiation of proteins in the solid state. I. Aggregation and disorganization of secondary structure in bovine serum albumin.

ALEXANDER, P., HAMILTON, L. D. G. AND STACEY, K. A. (Royal Cancer Hosp., London, Engl.). *Radiation Research 12*, 510–25 (1960).

Solid bovine serum albumin has been irradiated in the absence of oxygen with 2-Mev electrons. On irradiation the protein becomes insol. in water but remains sol. in salt solns. The loss of water soly. is assocd. with an increase in the average molecular wt. as detd. by light-scattering. The aggregates are not dissocd. by 4 *M* guanidine HCl or by the breaking of disulfide bonds. The fraction soluble only in salt solns. is rendered insol. in all solvents—except those which break disulfide bonds—by wetting the water. This is believed to be due to intermolecular bonds formed by disulfide exchange.

Turnover of protein and nucleic acid in soluble and ribosome fractions of non-growing Escherichia coli.

MANDELSTAM, J. AND HALVORSON, H. (Univ. of Wisconsin, Madison). *Biochim. et Biophys. Acta 40*, 43–9 (1960).

The stability of protein and of RNA has been examd. in the ribosome and sol. fractions of non-growing *E. coli*. In both fractions there is balanced degradation and re-synthesis of protein at about 5%/hr. The RNA of the ribosomes is also degraded at this rate but re-synthesized at only 1.5%/hr. In the sol. fraction the rate of nucleic acid synthesis exceeds the rate of degradation, so that there appears to be net transfer of material from the ribosomes to the soluble fraction. Degradation of ribosomes supplies much of the free amino acids and almost all of the ribonucleotides passing through the free pool during starvation.

Studies on the mechanism of fatty acid synthesis. VII. Biosynthesis of fatty acids from malonyl CoA.

GANGULY, J. (Univ. of Wisconsin, Madison). *Biochim. et Biophys. Acta 40*, 110–18 (1960).

Malonyl CoA can serve as the starting point of the synthesis of long chain fatty acids in animal tissues. The rate of formation of fatty acids from malonyl CoA is many times faster than acetyl CoA. TPNH and a particulate enzyme fraction are required for the synthesis of fatty acids from malonyl CoA. DPNH cannot replace TPNH efficiently in all tissues. The rate of formation of malonyl CoA from acetyl CoA seems to be the limiting step in fatty acid synthesis from acetyl CoA.

Level of sulfhydryl compounds in rat liver following feeding with goitrogenous and non-goitrogenous cabbage.

SEDLAK, J. (Slovak Acad. of Sci., Bratislava, Czech.). *Nature 186*, 982–3 (1960).

Rats were given a goitrogenous and non-goitrogenous variant of cabbage and total sulfhydryl compds. in liver measured immediately following death of the animals. Results showed that rats fed with goitrogenous cabbage store in the liver twice as much total sulfhydryl compds., (expressed in terms of glutathione) as do those fed on non-goitrogenous cabbage. It may be possible to use the estn. of total sulfhydryl compds. in the liver as a test of the goitrogenicity of vegetable foodstuffs.

Nutrition

Food intake, water intake and body water regulation.

LEPKOVSKY, S., ET AL. (Univ. of California, Berkeley). *Poultry Sci. 39*, 390–4 (1960).

Feeding chickens with or without water does not greatly influence their food intake, since other factors have greater effects. There is more water in the crop contents of chickens fed with water than in the crop contents of chickens fed without water. The intestinal contents of chickens fed with or without water have approximately the same percentage of water. Feeding with or without water does not affect the proteolytic activity of the intestinal contents. The rate of digestion is slower in chickens fed without water.

Experience with prolonged administration of intravenous fat emulsions.

COHN, I., ET AL. (Louisiana State Univ., New Orleans). *J. Lab. Clin. Med. 55*, 917–28 (1960).

The following reactions, previously reported in humans receiving intravenous fat emulsions, occurred in the exptl. animals: lethargy, vomiting, persistent lipemia, anemia, gastrointestinal bleeding, abnormal liver function, and deposition of intravenous fat pigment. Emulsion was administered to normal dogs without lethal results at a 40 ml./kg. dosage, whereas Lipomul I.V. was fatal when the dosage was greater than 30 ml./kg.

Megaloblastic anemia of infancy in Jamaica.

MACIVER, J. E. AND BACK, E. H. (University Coll. of the West Indies, Jamaica). *Arch. Disease in Childhood 35*, 134–45 (1960).

The literature on megaloblastic anemia of infancy is briefly reviewed with particular reference to its occurrence in association with protein malnutrition (kwashiorkor). Of the 50 cases in the series, 45 were given folic acid and 5 received vitamin B_{12}. Six of the folic acid treated cases died. The response to both folic acid and vitamin B_{12} in the remaining cases was excellent. No response to antibiotics was noted. Whether the main deficiency is of folic acid or of vitamin B_{12} is uncertain at the present time, although a deficiency of folic acid seems likely to be the more important.

The availability of bound nicotinic acid acid to the rat. 3. The effect of boiling maize in water.

CARPENTER, K. J., KODICEK, E. AND WILSON, P. W. (Rowett Research Inst., Aberdeen, Scotland). *Brit. J. Nutrition 14*, 25–34 (1960).

Boiling maize in tap water for 1 hr., combined with soaking for over 40 hr., did not result in any detectable release of nicotinic acid from its bound form; the resulting material did not cure rats suffering from nicotinic acid deficiency. Boiling maize for 5 hr., combined with previous soaking for over 20 hr., released only about 14% of nicotinic acid from its bound form, and the resulting maize prepn. had only a small curative or preventive effect, corresponding to the small amt. of nicotinic acid liberated. Maize treated with 1% lime-water had all its nicotinic acid in the free form and completely protected rats from, or cured them of, nicotinic acid deficiency.

DETERGENTS

Removal of fatty soil from glass. Electrolyte detergent-builder effect.

ANDERSON, R. M., SATANEK, J. AND HARRIS, J. C. (Monsanto Chem. Co., Dayton, Ohio). *J. Am. Oil Chemists' Soc. 37*, 119–21 (1960).

The mechanism of radio-tagged tristearin removal from a glass substrate by sodium tripolyphosphate (STP) is primarily one of preferential displacement. Tristearin removal by STP appears to be a competition for primary polar adsorption sites: being the more polar, STP displaces the soil. Continuous soil films are more slowly removed than spotty soil films because initially fewer accessible adsorption sites are available for displacement attack by STP. It is believed that emulsification of these heavy films occurs initially by the "stripping" or preferential displacement by STP of the

adsorbed monolayer at a site, followed by a rolling up of coherent soil along with the desorbed monolayer, resulting in a degree of emulsification.

ENGINEERING RESEARCH

Mechanisms by which ultrasonic energy effects transfer rates in liquid-liquid extraction.

WOODLE, H. A. AND VILBRANDT, F. C. (Virginia Polytechnic Inst., Blacksburg). *Am. Inst. Chem. Eng. J. 6*, 296–8 (1960).

Concurrent and spray-tower studies have been accomplished that illustrate the effects of ultrasonic insonation on transfer rates in liquid-liquid extn. Stage efficiencies and over-all transfer rates in the system toluene-methyl alcohol-water were detd. with and without insonation of the system at frequencies of 40 and 800 kc. Insonation was shown to have a positive effect on mass transfer in both concurrent and spray-tower operations. Insonation of the system provides for increased interfacial area between the two phases, removal of relatively stagnant liquid layers at the interface, and increased circulation currents within a single liquid phase.

FOOD AND FOOD TECHNOLOGY

Baking and Bakery Products

Volatile aromatic substances of rye bread. (In German).

ROTHE, M. *Ernahrungsforsch. 5*, 131–42 (1960).

Six amino acids (alanine, valine, leucine, isoleucine, methionine, phenylalanine) decompose with the formation of aldehydes when dry heated with xylose, and also under the prevailing conditions in rye bread during baking. These aldehydes are sepd. and identified by paper chromatography of the dinitrophenylhydrazones. Furfural and hydroxymethylfurfural are also present in rye bread crust, arising from the pentoses and hexoses.

Fats and Oils

The formation of polarographically reducible substances in autoxidizing lard.

KUTA, E. J. AND QUACKENBUSH, F. W. (Purdue Univ., Lafayette, Ind.). *J. Am. Oil Chemists' Soc. 37*, 148–51 (1960).

In a polarographic study of lard during the early stages of autoxidation at 55° and 100° C. 3 reduction potentials were observed, and curves were plotted from the diffusion current values at the corresponding half-wave potential to show the changes in concn. of each of the reducible substances with time. All three curves followed the general pattern of the peroxide value, determined titrimetrically. However, Curve A (E ½ = 0.15 v.) reached a maximum much earlier than either Curve B (E ½ = −0.68 v.)

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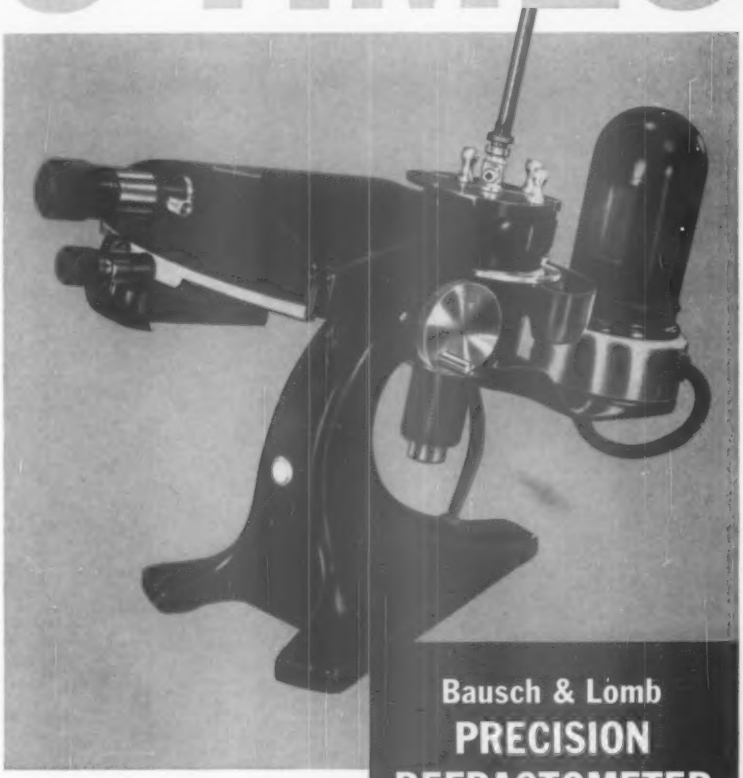

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or Curve C (E ½ = −1.10 v.). It is concluded that autoxidizing lard contains at least 3 types of primary "peroxidic" compds. and that these are not changed qualitatively by the presence of prooxidants and antioxidants.

Fish

Dicarbonyl compounds as components of fish odor.

MANGAN, G. F. *Comm. Fisheries Rev. 21*, 21–22 (July 1959).

The presence of dicarbonyl and α-hydroxy carbonyl compds. of four carbon atoms or less, are indicated in 2,4-dinitrophenylhydrazine derivs. of mixed carbonyl compds. prepd. from fresh and from frozen haddock. These compds. may be important odor and flavor components and possibly could be used as the basis for objective quality tests for fishery products.

Gelatin

The formation of fibrils from collagen solutions. 2. A mechanism of collagen-fibril formation.

WOOD, G. C. (Univ. of Leeds, Engl.). *Biochem. J. 75*, 598–605 (1960).

Fibril formation is regarded as the result of two processes: (a) nucleation, i.e. the aggregation of soluble collagen particles to form nuclei, the smallest aggregates capable of existing as a separate phase; (b) growth of the nuclei into fibrils by accretion of further soluble collagen particles. It is assumed that reaction of the soluble collagen particles with the surface of the growing fibrils controls the rate of growth and that the former process is proportional to the surface area of the precipitate and to the collagen concn.

Reversible transformation of gelatin to the collagen structure.

VEIS, A. AND COHEN, J. (Armour & Co., Chicago, Ill.). *Nature 186*, 720–1 (1960).

The dried acetone ppt. of a gelatin fraction recovered from bull-hide corium collagen was dissolved in 0.1 *M* KCl at 40° and the soln. clarified by centrifugation. A small, translucent gelatinous sediment was discarded. After setting to a firm gel in a cold room, the gel was placed in a 40° bath. Within 12 min. a fine turbidity developed and within 1 hr. floccules appeared which were collected by centrifugation. This ppt. was white and fibrous and typical collagen cross-striations appeared under the electron microscope. The fibers were readily sol. in 6.0 *M* urea. It is postulated that the inter-chain cross-linkages present in natural insol. collagen might be retained in the sol. degradation products.

An enzymatic examination of the structure of the collagen macromolecule.

VON HIPPEL, P. H., ET AL. (Naval Medical Research Inst., Bethesda, Md.). *J. Am. Chem. Soc. 82*, 2774–86 (1960).

The collagenase-catalyzed degradation of soluble ichthyocol has been examd. in order to obtain further insight into the structure and configuration of the collagen macromolecule. The kinetics of proteolysis were followed directly by pH-stat and colorimetric ninhydrin methods, and it is shown that below 27° the overall kinetics can be reduced to the sum of 2 concurrent reactions, both apparently first order in substrate concn. but differing markedly in rate. The results are interpreted in terms of a rigid, multistranded, inter-chain hydrogen-bonded structure for the collagen macromolecule in soln.; enzymatic cleavage of single strands leaves the particle relatively intact, but brings about a partial structural collapse by introducing points of increased flexibility.

The hydroxyproline content of porcine gelatin.

COURTS, A. AND KRAGH, A. M. (Brit. Gelatine and Glue Research Assoc., London). *Anal. Biochem. 1*, 88–95 (1960).

This note reports the hydroxyproline contents for 5 com. acid-processed pig skin gelatins and for 2 specially prepd. porcine gelatins, one from bone by an acid process and the other from skin by an alkaline process. The data are discussed in the light of results reported in the literature and conclusions are drawn with respect to the purity of mammalian collagens and gelatins.

IN MEMORIAM

John Clarkson Monk, chief engineer for Foremost Dairies, Inc., died July 16, 1960, at the age of 49. He joined Foremost nearly 15 years ago as project superintendent at the Golden State Green Street (San Francisco) plant. In 1954 he was promoted to chief engineer of the entire Foremost operation.

John Monk served on the California Manufacturers Association Industrial Waste and Smog Control committees, as well as the Golden Gateway Redevelopment Project. He belonged to the Institute of Food Technologists, California Dairy Institute, Commonwealth Club, California Society of Professional Engineers and the Stanford Club of San Francisco.

He is survived by his wife and two teen-age sons.

Edward M. Chace (retired), formerly chemist in charge of the U.S. Department of Agriculture, Fruit and Vegetable Chemistry Laboratory, died on August 9, 1960, at the age of 86, in Los Angeles. Mr. Chace started his scientific career in the Department of Agriculture in Washington, D. C., working on adulterated foods under Dr. Harvey Wiley, the well known father of our present food and drug law. Around 1908, Mr. Chace was transferred to Los Angeles where he set up the Fruit and Vegetable Chemistry Laboratory (now located in Pasadena) to work on the chemistry and technology of citrus fruits and fruit products. He was a recognized authority in the field of food chemistry. He published many scientific papers and was very active in the professional societies. Mr. Chace was awarded a 50 year service pin by the American Chemical Society, and was a recipient of the Appert Award of the Chicago Section of the Institute of Food Technologists. He is survived by his wife, two daughters and a son.

Harvey K. Murer, director of research at the General Foods Research Center in Tarrytown, N. Y., died as a result of an automobile accident Saturday, August 13, en route to Bergen, Norway.

Mr. Murer, who was 49 years old, was on vacation with his wife, Mrs. Clarissa Slichter Murer, and daughter, Miss Karen Ann Murer, both of whom are hospitalized in Voss Hospital, Voss, Norway, as a result of the accident. He lived in Saddle River, N. J. He also is survived by a brother, E. B. Murer of St. Louis, Missouri.

Mr. Murer joined General Foods Corporation in 1941 in the company's former Central Laboratories. He had been director of research since 1958.

Prior to joining GF, he served in research and teaching capacities at the University of Tennessee, Northwestern University and Washington State College. Mr. Murer was a graduate of Washington State College and also attended Harvard University and Northwestern University.

PERSONNEL

Mr. Paul V. Imes has recently been appointed to the new position of Technical Director of The Carey Salt Co. He has been a member of the IFT since 1944, and received an A.B. in chemistry and mathematics from Baker University, and an M.A. in physical chemistry from Kansas University. Among the responsibilities of his new position is that of providing technical counsel to Carey customers.

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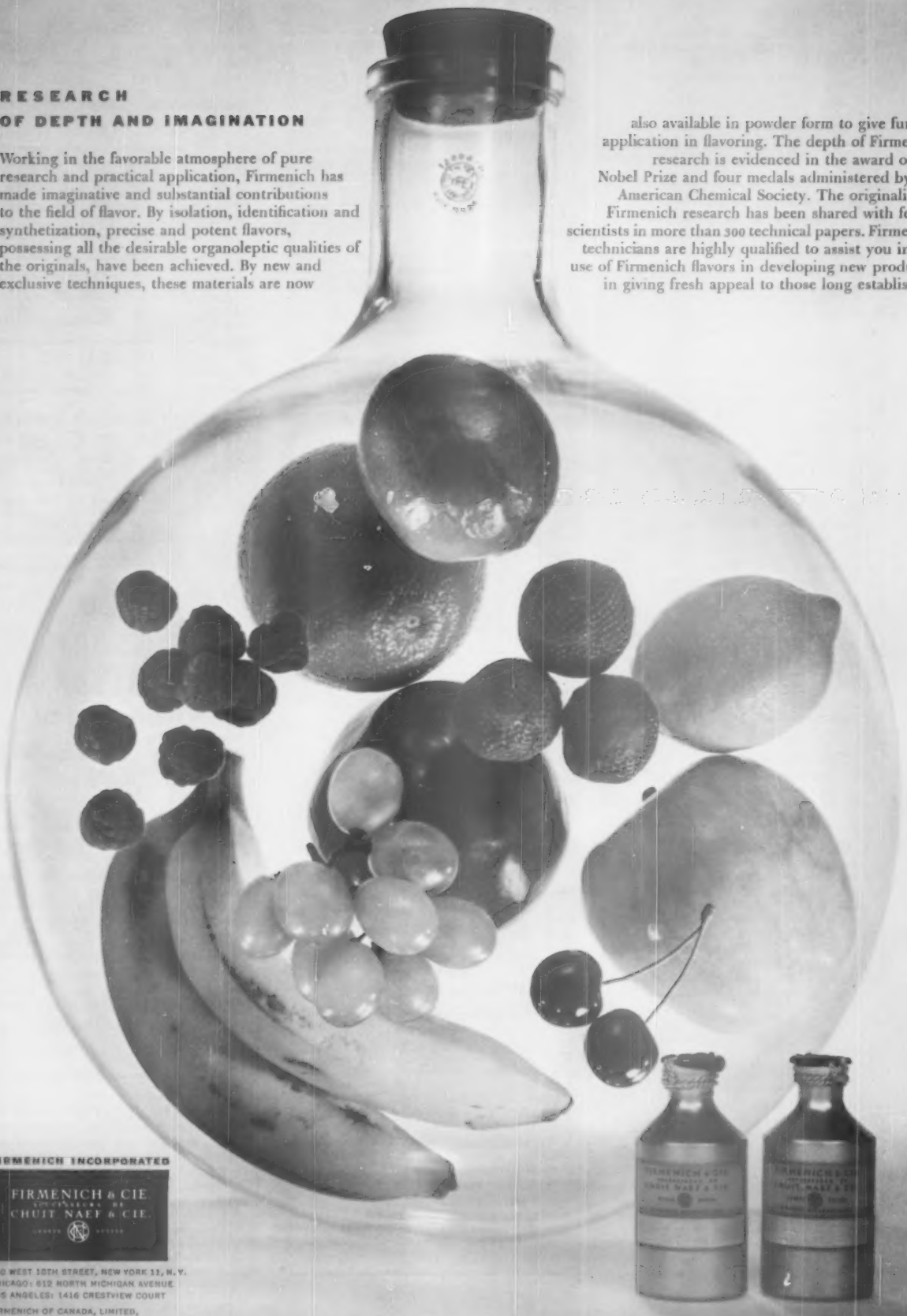

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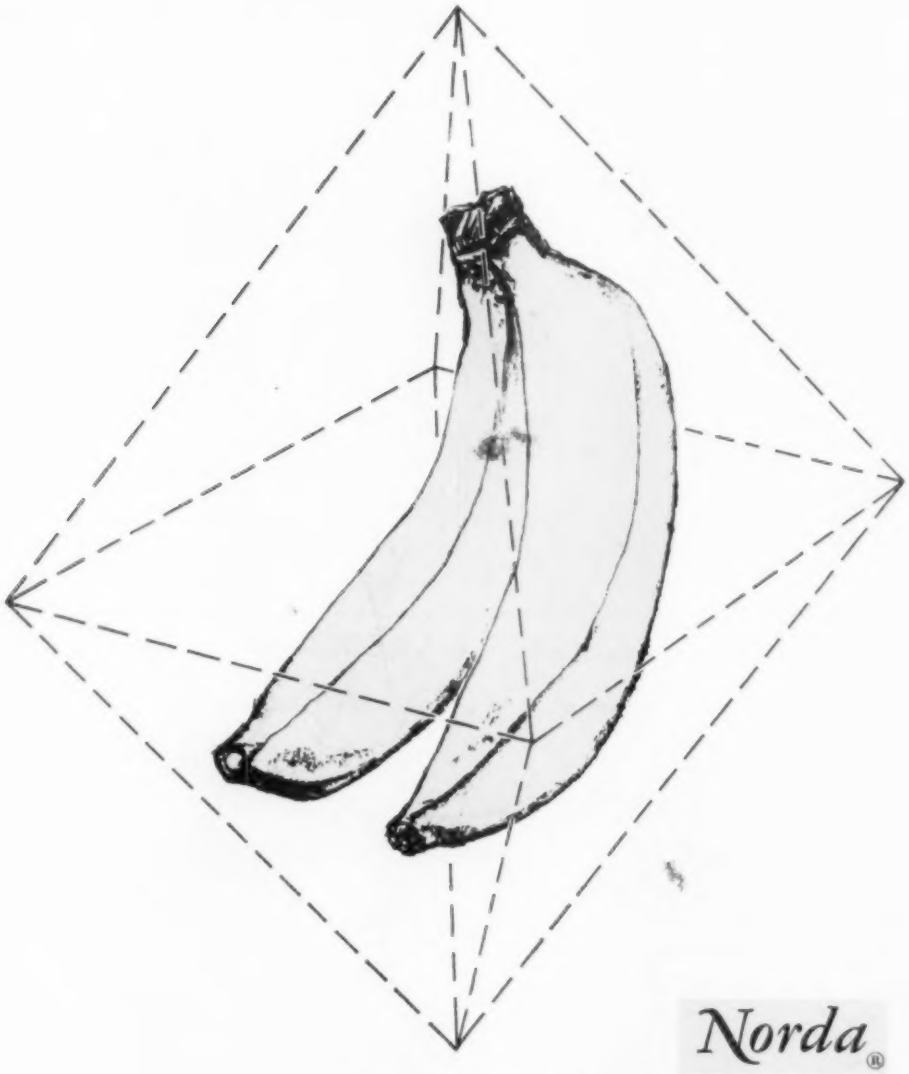

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